Newborn screening for cystic fibrosis in the Netherlands : the CHOPIN study

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The CHOPIN study

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Als je benen bewegen dan loop je of ren je.
Merel Vernooij, 2,5 jaar

Voor Merel, Joppe en Floor
Voor Joris

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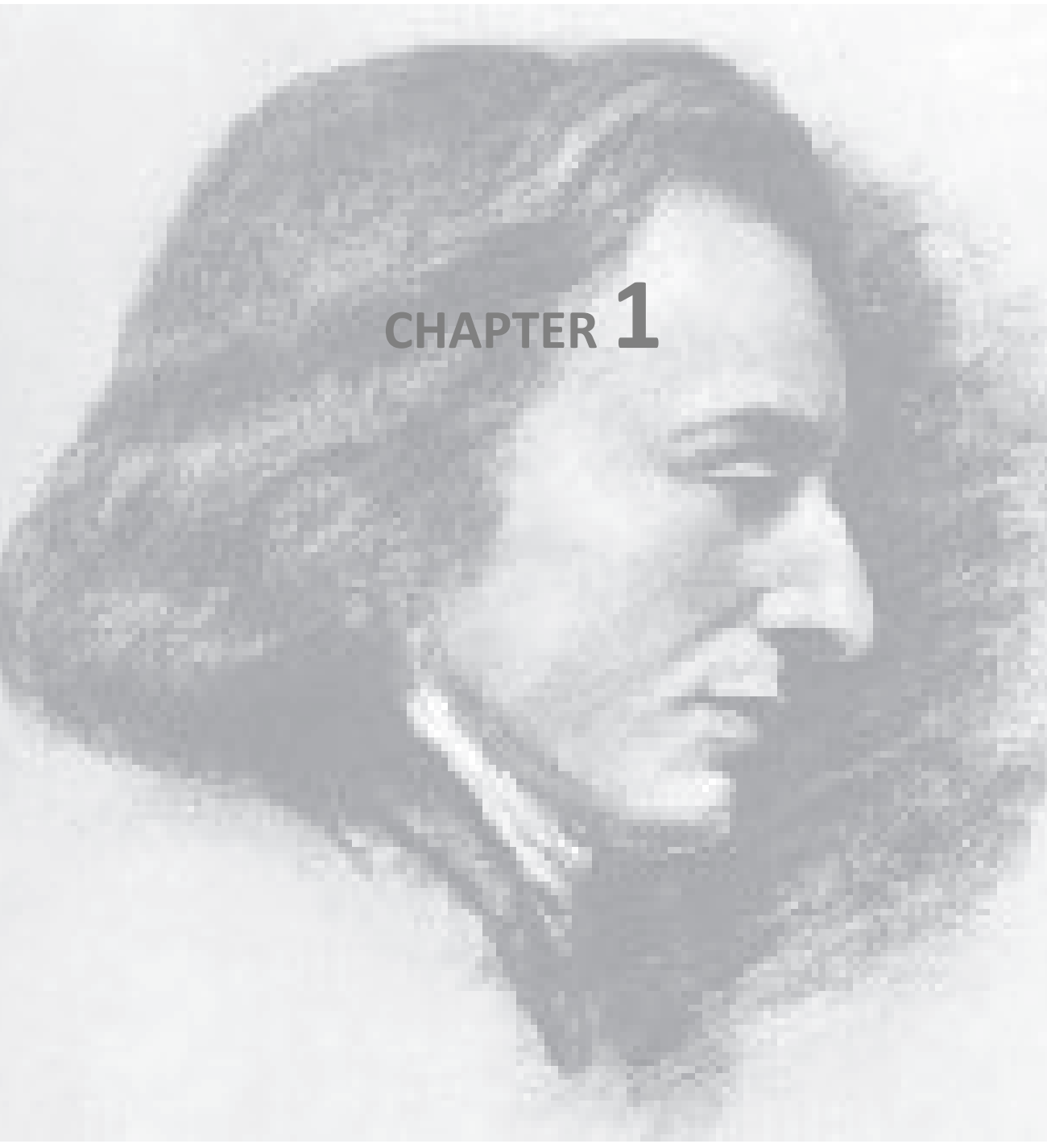

CHAPTER 1

Frederic Chopin, the famous 19th century composer is thought to have suffered from cystic fibrosis. Chopin had a length of only 170 cm, and weighed less than 45 kg. He suffered from recurrent respiratory infections from early childhood and chronic cough with mucus production. Chronic diarrhoea and fat malabsorption would fit the probability of pancreas insufficiency. Chopin died at the age of 39 years.[1]

General introduction

General introduction

Cystic fibrosis

Cystic fibrosis (CF) is one of the most common inherited diseases in the Netherlands and other western countries, with around 1 in 4750 newborns affected in the Netherlands.[2] CF is caused by pathogenic mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene that encodes for a chloride transporter protein in the membrane of epithelial cells. CF has an autosomal recessive inheritance, and over 1900 CFTR mutations are known.[3]

CFTR mutations can be classified in five different classes, depending on the effect of the mutation on production, transport and function of the chloride channel.[4,5] Class I mutations cause a premature stopcodon, mRNA is not translated and CFTR is not produced. Class II mutations result in defective transport of the CFTR to the cell surface. The most prevalent CFTR mutation, F508del, belongs to this group. Class III are gating mutations, the channel reaches the membrane but does not open. In Class IV mutations the CFTR protein reaches the cell membrane but the channel is too narrow to function well enough.
Class V mutations are very rare, they cause splicing defects leading to less mRNA being processed and therefore to a reduced number of well functioning channels.[6] Classic CF is caused by class I to III, and class IV and V mutations lead to non-classic CF.

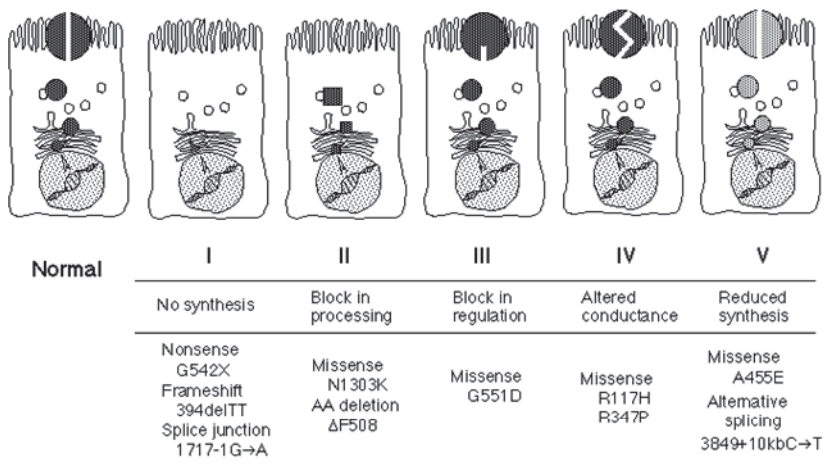

Figure 1.1 Five different classes of CFTR mutations.[7]

The **CFTR** consists of two membrane spanning domains (MSD), two nucleotide binding domains (NBD) and a cytoplasmic regulatory domain. The NBD is responsible for ATP hydrolysis which provides energy for channel activity. The two MSD provide a channel pore for chloride transport whereas the regulatory domain has an inhibitory or stimulating effect on chloride transport. Dysfunction of this transporter protein leads to limited transport of chloride through the cell membrane, which in turn leads to the formation of thick and sticky mucus in various organs. The affected organs include the upper and lower airways, pancreas, liver, intestines, sweat glands and vasa deferentia in males.

The **thick and sticky mucus** causes obstruction of the small airways and an increased susceptibility to bacterial infections which are often symptomless in the first phase. Infection of the lower airways is always accompanied by an exuberant inflammatory reaction, leading to progressive destruction and often to an increase in complaints and respiratory symptoms like recurrent coughing, phlegm, and breathlessness. The so-called exacerbations of CF lung disease are clearly related to (the decline in) lung function and affect the prognosis. Obstruction of the pancreatic ducts cause destruction of the pancreas resulting in malabsorption and malnutrition because of digestive enzyme deficiency. Infertility is a problem in male subjects and is caused by agenesis of the vas deferens. Sweat glands produce sweat with a high concentration of sodium and chloride.

The gold standard test to confirm the diagnosis CF is the **sweat test**. A sweat chloride concentration of more than 60 mmol/l in combination with a characteristic clinical picture is pathognomic for CF.[8] Sweat tests can be performed from a gestational age of 38 weeks and a birth weight of 2000 grams and at the postnatal age of two weeks.[9] Performing a sweat test in a newborn is a challenge, as newborns have a low sweat rate, resulting in a high failure rate.

The **first symptoms of CF** in infants often are feeding difficulties, failure to thrive and recurrent airway infections. Those complaints often occur in young infants, which leads to delay of the CF diagnosis. However, about 50% of the Dutch CF patients was diagnosed before the age of five months.[10] In about 13-17% of the cases the diagnosis is made early after birth because of a meconium ileus.[11] Another possibility for early detection is newborn screening.

Newborn screening for cystic fibrosis

There has been a long debate about the benefits and disadvantages of newborn screening for CF (NBSCF). It took some time for governments to get convinced of the

benefits of NBSCF, but nowadays it is widely accepted worldwide.[12,13] An early diagnosis leads to normal growth and a better nutritional status by treatment of the pancreas insufficiency with digestive enzymes and vitamin supplements.[14-17] There is observational evidence that an early diagnosis leads to a better preservation of lung function until adulthood.[18-20] Early treatment of infections may prevent lung damage and will lead to less and shorter hospital admissions and less invasive therapies.[21-23] There are data suggesting that NBS may improve survival.[19,20,24,25]

However, next to advantages, NBSCF also has disadvantages:

1) False-positive results. A big problem in daily practice is the arousal of parents by a positive screening test when afterwards their child turns out to be healthy. The time between the positive screening result and confirmation or exclusion of the diagnosis is very stressful for the parents.[26,27]
2) False-negative test results. The situation in which an infant with CF has a negative screening test and is detected because of clinical symptoms at a later age, is a source of parental stress and medical delay in diagnosis and treatment.
3) Another problem of newborn screening is detection of phenotypically healthy babies with an equivocal diagnosis. These infants have two CFTR mutations, of which at least one with unclear clinical significance, and a normal or equivocal sweat test.[28] Because the clinical prognosis is uncertain, the parents of these infants often cannot be completely reassured.
4) NBS may also reveal healthy carriers of CF, which may have consequences for the child's future, the parents and their family.[29] Although disclosure of the carrier status to the parents may generally be evaluated as an advantage of newborn screening, it may be experienced as a disadvantage for those parents not wanting to know the carrier status of their child.

NBS should lead to a substantial health benefit by early detection and treatment. In 2005 the Health Council of the Netherlands advised to expand the Dutch NBS program with 14 metabolic disease and sickle cell anaemia.[30] The Health Council of the Netherlands decided that the benefits for NBSCF outweighed the disadvantages, but that the available screening tests were not sufficiently reliable. The Health Council advised to implement screening for CF only on the condition that a better screening test would become available in the Netherlands with less disadvantages and a higher specificity than the existing international programs.

The paradox is that although NBSCF is now widely accepted, there is no universally accepted or ideal screening strategy.[13] A survey of screening programs in Europe described 26 different screening strategies.[31]

All programs start with measuring the concentration of immunoreactive trypsinogen (IRT) in dried blood spots. The second tier is either a limited CFTR mutation analysis or a repeat measurement of the IRT concentration at the age of 4-6 weeks.[32]
Disadvantages of those programs are a high false-positive rate and detection of carriers and equivocal diagnosis. In 2005, pancreatitis-associated protein (PAP) was described as a possible second tier in NBS for CF. Measurement of IRT as well as PAP in dried blood spots may lead to a specific and sensitive screening program.[33,34] A different IRT-PAP protocol was used in Germany in 2009, i.e. a 99th percentile IRT cut-off level and a PAP cut-off level of 1.6 µg/l.[35] Screening with IRT-DNA followed by sequencing of the CFTR gene in all samples with a single CFTR mutation may be an alternative strategy. In this approach the screening test is only considered to be positive if two mutations are identified.[36] In California, a comparable screening protocol is in use since 2007, but in this protocol infants with a single mutation are also referred for a sweat test.[37]

There was a need for novel screening strategies to retain the benefits of NBSCF but decrease the number of false-positive results, detection of carriers and equivocal CF diagnosis. In this thesis two novel strategies for NBS for CF were investigated: IRT-PAP and IRT-DNA-sequencing, and a third combined strategy was analysed afterwards (IRT-PAP-DNA-sequencing). Although earlier studies has shown that screening is cost-effective, the cost-effectiveness is not known for these novel strategies. Moreover, it is unclear whether PAP levels in newborns are affected by sex, gestational age, birth weight, blood transfusion or timing of the blood sampling. It is important to know this possible influence before PAP is used as a reliable new test method in NBSCF.

After a positive screening test for NBSCF, the diagnosis must be confirmed by a sweat test in a CF centre. The success rate of the gold standardsweat test methods (QPIT and Macroduct) decreases rapidly under 6 weeks of age, which is a problem. A new test method, the Nanoduct, is available but it is unknown whether this instrument increases the success rate and is reliable enough for (exclusion of) a CF diagnosis.

Without screening, the diagnosis in the Netherlands was made because of clinical symptoms or a family history of CF. In the 1990's, the median age at diagnosis was around the age of 14-18 months. At that time NBSCF had a long term effect until the age of 10 years.[25] Nowadays, the median age of diagnosis is five months.[10] It is unclear if NBSCF still can improve the clinical condition and prognosis of patients with CF in the first years of life in the Netherlands.

Aims of the study

The aims of the Cystic fibrosis Heel prick amOng a newborn Population In the Netherlands (CHOPIN) study were:

- To evaluate the test properties (sensitivity, specificity and positive predictive value) of two novel screening strategies for newborn screening for cystic fibrosis: IRT-PAP and IRT-DNA-sequencing.
- To determine the influence of sex, gestational age, birth weight, blood transfusion, and timing of the heel prick on the pancreatitis-associated protein (PAP) concentration in the blood of newborns.
- To investigate the cost-effectiveness of IRT-PAP and IRT-DNA-sequencing screening strategies in newborns in the Netherlands.
- To assess whether a false-positive screening test result will lead to parental stress and concern and if these emotions still exist after six months.
- To assess the opinion of the parents about the disclosure of the carrier status in NBS for CF.
- To explore if the Nanoduct, a sweat test system especially developed for young infants, can be used to confirm or exclude CF after a positive newborn screening test.
- To compare the clinical condition of infants with CF after NBS with children discovered by clinical symptoms.

Outline of the thesis

Chapter 1 presents a general introduction on the disease, symptoms, aetiology, pathogenesis and screening. **Chapter 2** reviews the benefits and disadvantages of NBS for CF in general. **Chapter 3** describes the main results of the CHOPIN study in which two novel screening strategies are compared: IRT-PAP and IRT-DNA-sequencing. A post hoc analysis of a third strategy was added; consisting of IRT, followed by PAP, DNA mutation analysis and sequencing. **Chapter 4** shows the influence of gestational age, birth weight, sex, blood transfusion and timing of the heel prick on the level of PAP in the blood of newborn infants. Data on a cost-effectiveness of four different NBS programmes are provided in **Chapter 5**. **Chapter 6** describes parental feelings of anxiety and concern after a false-positive screening test result and six months later. Both questionnaires and interviews were used for this purpose. In **Chapter 7** we show the results of the focus groups with pregnant couples and answer to the question whether or not they want to be informed about the carrier status of their child if this would be detected by NBS. **Chapter 8** shows the difference in clinical symptoms and growth between a population detected after NBS compared to a group of CF children discovered by a clinical diagnosis. In **Chapter 9** the Nanoduct is compared with the Gold standard sweat test methods (QPIT and Macroduct) in 108 children referred to a CF centre because of a positive NBS test for CF. **Chapter 10** contains the general discussion and **Chapter 11** the summary in English and Dutch.

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CHAPTER 2

Benefits and disadvantages of newborn screening for cystic fibrosis

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Abstract

During the past decade, newborn screening for cystic fibrosis (CF) has been introduced in many countries. However, the disadvantages associated with newborn screening for CF hamper its introduction in other areas. Within the routine heel-prick programmes implemented around the world, there is no other disease that elicited such a debate about whether or not to include it in these programmes as CF.

The reasons for this debate are manifold: the diagnosis of CF is not as simple as it looks on first sight; a robust screening test suitable for universal use does not exist; and the effects of early management on the outcome are doubted by many. The improvement in survival of patients with CF observed during the last few decades has been attributed to an improvement of the traditional therapies for CF. Examples include: pancreatic enzyme replacement therapy, high caloric intake and supplementation of fat-soluble vitamins for CF gastrointestinal disease; early eradication of airway infection with Pseudomonas aeruginosa (inhalation therapy with antibiotics when the infection becomes chronic); and improved therapies for mucocilliary clearance using dornase alfa and hypertonic saline. However, therapies aimed at the basic defect have not yet been developed.

Newborn screening for CF allows early diagnosis, often when the child is still asymptomatic, or only has a few symptoms related to poor growth but no pulmonary problems. However, the evidence that starting treatment before respiratory problems have arisen leads to a better outcome on the long term is not very strong. There is ample evidence that in the first decade of life, CF patients identified by screening have a better clinical condition than patients diagnosed clinically. In this article we shall discuss the most common arguments for and against newborn screening for CF.

Benefits of newborn screening for CF

The best arguments for screening can be found when observing cohorts of patients with Cystic Fibrosis (CF) diagnosed clinically in areas where no newborn screening (NBS) programme for CF is running. Newborn screening leads to diagnosis at a very young age. In most countries without newborn screening, the median age of diagnosis is one year, and when patients with a meconium ileus are excluded, the median age is even higher; for example, in Canada, the median age at diagnosis is two years and in Sweden, it is six months,[1,2] while with a newborn screening programme, the median age at diagnosis decreases to 29 days.[3] In regions without screening, clinical symptoms finally lead to the diagnosis. Observational data from Canada show that 81% of clinically diagnosed CF patients have respiratory symptoms, many show failure to thrive (37%) and reduced serum concentrations of fat-soluble vitamins due to malabsorption, and 95% have gastrointestinal complaints at the time of diagnosis.[1] The mean head circumference of patients with a vitamin E deficiency was significantly lower, at the 32nd percentile (95% confidence interval (CI) 24–41%), than that of patients with a normal vitamin E concentration, of whom the mean head circumference was at the 63th percentile (95% CI 47–78%).[1]

Hospital admissions are not uncommon during the period in which children develop symptoms: 79% of the children were hospitalised, with a mean length of stay of five days (range 1–30 days) in a tertiary care hospital before the diagnosis CF was made. Some children already had severe bronchiectasis at the time of diagnosis.[4] Many of these problems associated with a late diagnosis can be prevented. Evidence for beneficial effects of screening has been reported in two randomised clinical trials,[5-7] in analyses from large clinical databases[8-10] and from a few cohort studies.[11-16]

Treatment of pancreatic insufficiency

Gastrointestinal problems in CF are mostly related to pancreatic insufficiency. Most patients with CF are pancreatic insufficient at birth or develop pancreatic insufficiency during the first year of life.[17] With an early diagnosis, pancreatic disease can be treated from the start with pancreatic enzyme replacement therapy.

Growth

In a randomised clinical trials,[5,7] a large clinical database[8] and four observational cohort studies, a significant improvement of height and weight was observed compared with clinically diagnosed CF.[10,13,15,16] With an early diagnosis, children with CF can maintain a growth pattern that approximates that of healthy children.[5]

Nutritional deficiencies

Malnutrition by protein deficiency and significant morbidity due to deficiencies of fat-soluble vitamins have been described in clinically diagnosed patients, and newborn screening can prevent this morbidity.[18,19] Vitamin deficiency can occur very early in life. Many infants diagnosed by screening already show low concentrations of vitamin A (60%), D (37%) and E (16%), underlining the need for early diagnosis and start of treatment.[20] There is indirect evidence that early malnutrition and nutritional deficiencies early in life are related to lung growth. Patients with CF with pancreatic insufficiency who achieved early growth recovery within two years of diagnosis had fewer cough symptoms, higher lung function and better chest radiography scores at six years of age.[21]

Lung function

A randomised clinical trial showed better chest radiography scores early in life but at a later age, no difference in lung function between the screened cohort and the clinically diagnosed patients was found.[22] This may be related to the fact that the screened cohort had earlier P. aeruginosa colonisation than the clinically diagnosed cohort. In all cohort studies, significantly better chest radiography scores were observed in the screened cohorts. Cross-sectional studies with data derived from the US database found better lung function in screened patients aged 6–10 and 11–20 years.[19] Cohort studies from the Netherlands and Australia showed better lung function until 12 and 15 years of age in the screened cohorts, respectively.[11,14] In the UK database, no difference in lung function was observed between screened and non-screened cohorts, but the screened cohort needed significantly less treatment.[23] There is also indirect evidence that early diagnosis and treatment are related to better lung function, as a cohort study showed that in sibling pairs, the younger siblings show a significantly better lung function at adult age.[24] Moreover, while recruiting patients of 12 years of age for the Early Pseudomonas Infection Control (EPIC) study, it was found that patients with a diagnosis through newborn screening had a significantly better forced expiratory volume in 1 second % predicted than clinically diagnosed patients.[25] It is possible to eradicate P. aeruginosa during the first infection in practically all patients, but practically impossible to eradicate P. aeruginosa when the infection has become chronic. Pseudomonas can colonise the airways of young infants with CF in the first months of life without causing symptoms but leading to infection, inflammation and structural lung disease.[26] The observation that an early diagnosis does not change the time to first acquisition of Pseudomonas is another argument for the need for NBSCF: with an early diagnosis and thorough monitoring of airway infection, it is possible to

start eradication treatment of the first infections with pathogenic micro-organisms, which can postpone chronic infection for a long time.

Survival

In one randomised clinical trial, a higher risk was found for an early death in the clinically diagnosed patients;[6] a systematic review also reported a lower mortality risk in screened cohorts.[27]

Hospital admissions and burden of care

Fewer hospital admissions were found in one randomised clinical trial[6] and in two cohort studies.[15,19] In the UK database cohort study, less therapy was needed, and improved growth and reduced morbidity were found in the screened cohort.[23]

The opinion of parents

Parents prefer an early diagnosis even when their child has an untreatable disorder in order to prevent a long diagnostic odyssey.[28] In a recent study in Sweden, where currently no NBSCF takes place, parents favoured screening.[29] Parents of children with CF with a diagnosis within the first 3 months of life had more confidence in the medical profession and less negative feelings than parents of children with a late CF diagnosis.[30] An early diagnosis of a genetic disease such as CF also renders the opportunity that parents receive genetic counselling, which is important for further family planning. In some countries with longstanding newborn screening programmes for CF, the prevalence of CF at birth has been steadily diminished during the last 20 years.[31]

Research needs

Present treatment for CF cannot reduce the increased susceptibility of CF airways to bacterial infection, and once the airways have become infected it is extremely difficult to eradicate the invading micro-organisms. There is a need for research that can elucidate the process of bacterial infection in the CF airway, which may lead to improved treatment options. Clinical trials that investigate interventions in young infants without pulmonary involvement can only be performed when infants with CF are identified early in life, before pulmonary involvement, i.e. by newborn screening.

Economic arguments

Newborn screening for CF seems cost effective and can lead to cost savings, as costs of treatment can be reduced by 10%.[32,33] A more recent analysis assumed that the costs of screening, diagnosis and treatment in the first 3 years of life are about 71% of the

costs of diagnosis and treatment without a newborn screening programme, which means that NBSCF can lead to savings.[34]

Disadvantages of newborn screening for CF NBSCF

Effect on parents due to abnormal results of newborn screening tests

Most parents experience high levels of emotional stress during their wait for further diagnostic testing, mostly the sweat test. It may cause depressive symptoms that vary depending on their perceptions of how likely it is their child has CF.[35] A good protocol for handling abnormal screening test results with a minimum waiting period between the notification of the abnormal test result and the sweat test, and parental education may reduce much of the parental stress.[36]

False-positive tests

Practically all screening programmes lead to abnormal results that, during the following diagnostic process, appear to have been a false alarm. Such false alarms often lead to parental stress and anxiety. However, in the long-term, when the child appears to be healthy and with good parental education, parental anxiety levels do not differ from parents of children who did not get such an alarm.[36]

Identification of carriers

Screening approaches that include CF transmembrane conductance regulator (CFTR) mutation analysis also identify healthy infants carrying one CFTR mutation. An advantage of this finding may be that parents are offered genetic counselling, and occasionally both parents may turn out to be carriers. This can be important information for further family planning. However, in most cases, only one parent will be identified as a carrier, which can lead to anxiety and stress. From the child's perspective, the knowledge of being a carrier is not of direct and immediate benefit. Moreover, as the child could not decide whether or not (s)he wished to be tested, this can be considered as a violation of the ''right not to know''.

Inconclusive results of NBSCF

In most screening programmes, quite a number of infants are found by newborn screening in whom the diagnosis CF cannot be confirmed nor excluded, because they do not meet the diagnostic criteria for CF. Mostly, they have elevated immunoreactive trypsinogen (IRT) concentrations, normal or borderline sweat chloride concentrations, and carry one or two CFTR mutations with unclear clinical consequences. Some of these infants will develop a complete or partial CF phenotype later in life, but many

will never develop any symptoms. The uncertainty about prognosis is difficult for genetic counselling and requires that such infants get a regular further follow-up at CF centres. This may have a lifelong impact on the child and his or her family.

Risk for early colonisation with Pseudomonas aeruginosa

Various studies showed less chronic airway infection with P. aeruginosa.[9,12,19,23] However, in one randomised clinical trial, infants identified by screening developed colonisation and chronic infection with P. aeruginosa at an earlier age than clinically diagnosed patients with CF.[22] This may relate to cross-infection. The finding underlined the fact that patients with CF have an innate increased susceptibility for early airway colonisation with opportunistic micro-organisms, such as Staphylococcus aureus and P. aeruginosa. Infants identified by newborn screening will mostly visit CF clinics at an earlier age than clinically diagnosed patients; special hygienic measures to prevent cross-infections from older CF patients should be in place in every CF clinic.

Risk of ethnic discrimination

The use of a screening strategy including CFTR mutation analysis carries the risk that very rare mutations will not be identified. In countries with large multi-ethnic populations, this may lead to more missed cases in infants originating from non-Western countries. A European survey of mutations in CF patients of North-African or Turkish descent showed that only 63% of patients of Turkish origin would be identified by newborn screening.[37] It may be possible to compensate by using procedures such as a second IRT test if the first level was very high, or by performing DNA scanning of the CFTR gene in samples with very high IRT concentrations without mutations in the common panel. Many of the hazards associated with running a newborn screening programme for CF can be reduced. Notification of abnormal test results should lead to prompt referral and diagnostic testing according to an established protocol with solid parental information. Parents should be directed to safe and reliable sources of information on internet. Novel screening strategies can reduce the number of false positives and equivocal diagnosis.[34] Infants with a CF diagnosis should not be exposed to older patients with CF to prevent cross-infection with P. aeruginosa.

Discussion

Most arguments that favour NBSCF seem to be dependent on the management and treatment that can be given to the child with CF identified by screening. NBSCF leads to diagnosis before lung disease and malnutrition have appeared. Whilst after a clinical diagnosis, all efforts are primarily directed to treating disease in the affected organs,

after a diagnosis by newborn screening, the main challenge is to keep the infant as healthy as possible. This should lead, and often does lead, to a change to proactive, preventive medicine rather than reactive damage limitation. Recent evidence supports the notion that very early treatment of pulmonary infection and inflammation is a key factor for further improving prognosis of patients with CF and further research in this area is urgently needed. Most arguments against screening seem to be related to the imperfect properties of the available screening tests for CF; therefore, as tests improve, most of the arguments against screening will disappear. Since May 2011, the Dutch newborn screening programme has been extended with screening for CF after a pilot study assessing the characteristics of two novel screening strategies.[34,38] The screening test now in use in the Netherlands differs from screening tests used in other countries: it has a very high specificity and positive predictive value, and detects only a very small number of carriers and patients with an equivocal diagnosis.[34] With this screening programme, most of the arguments against screening, as discussed in this article, are no longer relevant.

Finally, costs of screening cannot be used as an argument against newborn screening for CF. Although the savings of an early diagnosis of CF are not as clear cut as in newborn screening for congenital hypothyroidism or phenylketonuria, NBSCF and treatment appear to cost less than a clinical diagnosis and treatment.[24]

Conclusion

The benefits of NBSCF are now generally accepted. The benefits of NBSCF amply compensate for the disadvantages. Moreover, NBSCF is cost-effective and there is growing evidence that it leads to cost savings. Many of the disadvantages of NBSCF are reduced to practically nil with the novel screening strategy that has recently been introduced in the Netherlands.

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CHAPTER 3

Novel strategies in newborn screening for cystic fibrosis: a prospective controlled study

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Thorax 2012; 67:289-95

Abstract

Background

Newborn screening for cystic fibrosis (NBSCF) is included in many routine newborn screening programs, but current strategies have considerable drawbacks such as false-positive results, equivocal diagnosis and detection of carriers.

Aim of the study

To assess the test performance of two new screening strategies in NBSCF.

Methods

In 2008 and 2009, CF-screening was added to the routine screening program as a prospective study in part of the Netherlands. Two strategies were performed in all newborns. In the first strategy, concentrations of immunoreactive trypsinogen (IRT) and pancreatitis-associated protein (PAP) were measured. In the second method, samples with IRT ≥60 μg/l were analysed for 35 CFTR-mutations, followed by sequencing when a single mutation was detected. Tests were positive only with two identified CFTR mutations.

Results

145,499 infants were screened. The IRT-PAP approach showed a sensitivity of 95.0% with a specificity of 99.897%, and a PPV of 12.3%. Test properties for the IRT-DNA-sequencing strategy were respectively 100%, 100%, and 64.9%. Combining both strategies (IRT-PAP-DNA-sequencing) led to a sensitivity of 95.0%, a specificity of 100%, and a PPV of 87.5%.

Conclusion

In conclusion, all strategies performed well. Although there was no statistical significant difference in test performance, the IRT-DNA-sequencing strategy detected one infant that was missed by IRT-PAP(-DNA-sequencing). IRT-PAP may be the optimal choice if the use of DNA-technology must be avoided. If identification of carriers and equivocal diagnosis is considered an important disadvantage IRT-PAP-DNA-sequencing may be the best choice.

Introduction

Newborn screening for cystic fibrosis (NBSCF) is widely accepted, but there is no universally accepted screening strategy.[1] A survey of screening programs in Europe, described 26 different screening strategies.[2]

All programs start with measuring the concentration of immunoreactive trypsinogen (IRT) in dried blood spots. The second tier is either a limited cystic fibrosis transmembrane regulator (CFTR) gene mutation analysis or a repeat measurement of the IRT concentration at the age of 4-6 weeks.[3] Protocols using IRT alone or IRT-IRT have a high false positive rate.[4] The major drawback of using CFTR mutation analysis is the high number of identified healthy carriers and cases with an equivocal diagnosis.[1,5]

In 2005, pancreatitis-associated protein (PAP) was described as a possible second tier in NBSCF. Measurement of IRT as well as PAP in dried blood spots may lead to a specific and sensitive screening program.[5,6] A different IRT-PAP protocol was used in Germany in 2009, i.e. a 99th percentile IRT cut-off level and a PAP cut-off level of 1.6 µg/l.[7]

Screening with IRT-DNA followed by sequencing of the CFTR gene in all samples with only one CFTR mutation may be an alternative strategy. In this approach the screening test is only positive when two mutations are identified.[8] In California a comparable screening protocol is in use since 2007, but infants with a single mutation are also referred for a sweat test.[9]

We hypothesized that these two novel screening strategies (IRT-PAP and IRT-DNA-sequencing) may lead to a similar sensitivity as current newborn screening strategies, but with a higher specificity, less carrier detection, and less equivocal diagnoses. The study aim was to compare the test performance of these two strategies in a large population of newborns in the Netherlands.

Methods

Study population

In the Netherlands all newborns are included in the routine newborn screening program, unless the parents refuse participation (opting-out procedure). The Dutch newborn screening program consists of 17 diseases (congenital adrenal hyperplasia, congenital hypothyroid disease, sickle cell disease and 14 metabolic diseases; www.rivm.nl/hielprik). Five laboratories spread over the country perform newborn screening (NBS), and receive heel prick samples from five designated areas. Two laboratories, the reference laboratory (RIVM) and the laboratory of the region South

East (Clinical Chemical Laboratory, St. Elisabeth Hospital, Tilburg) participated in the study.
All heel prick samples received in the two participating laboratories were tested with both screening strategies in 2008 and 2009, unless the parents refused the screening for cystic fibrosis (CF). Parents were informed about the screening for CF by a leaflet, available in ten languages.

Screening protocols

Figure 3.1 shows a flow chart of both screening strategies. The IRT-PAP protocol consisted of measurement of IRT and PAP in all samples. A positive result was defined as a combination of IRT ≥100 μg/l and PAP ≥1.6 μg/l, or IRT ≥60 μg/l and PAP ≥3.0 μg/l, as described before,[6] and corrected according to the publication at www.isns-neoscreening.org/htm/news in March 2011. In the IRT-DNA-sequencing protocol, an elevated IRT (≥60 μg/l) was followed by a DNA mutation analysis consisting of 35 mutations. When only one mutation was detected, DNA sequencing was performed. In this strategy the screening test was positive when two mutations were detected. All newborns with a positive screening result with one or both strategies were referred to a CF-centre for a sweat test to confirm or to exclude the diagnosis.

Definitions

The diagnosis of CF was confirmed by a sweat chloride concentration of ≥ 60 mmol/l. If this was not possible or the sweat test failed, the diagnosis can also be confirmed by two CFTR mutations, and/or a meconium ileus and/or positive family history.[10,11]
An equivocal diagnosis was defined according to international standards as an equivocal sweat test result (chloride 30-60 mmol/l) or a normal sweat test result (chloride <30 mmol/l) on two occasions in a newborn with two CFTR mutations of which one or both have unclear clinical consequences.[11] All infants with an equivocal diagnosis were regularly seen at the CF-centres during the first year of life. In the IRT-PAP strategy CF was excluded when the chloride concentration was below 30 mmol/l. In the IRT-DNA-sequencing strategy newborns with a single CFTR mutation after DNA-sequencing were considered as healthy carriers and screen-negative.

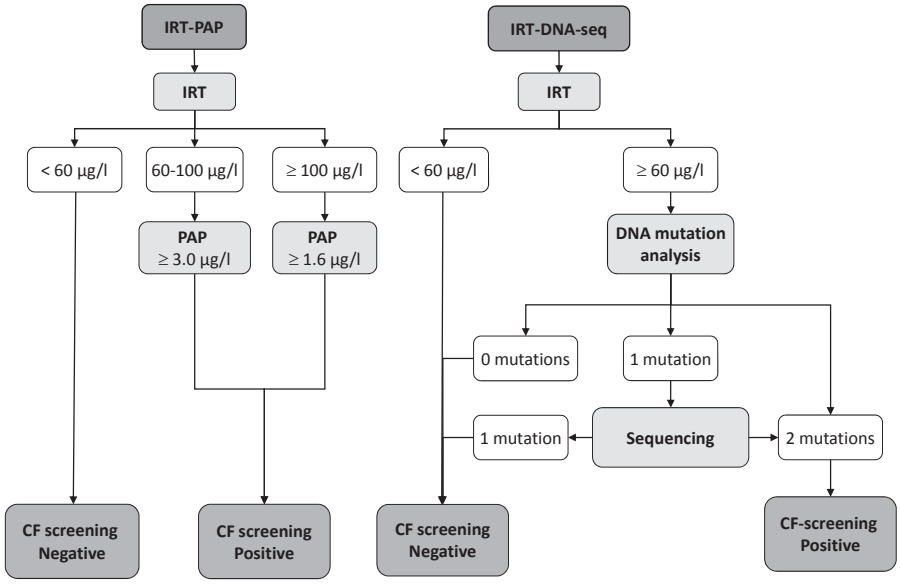

Figure 3.1 Flowcharts of both screening strategies for newborn screening for cystic fibrosis: IRT-PAP and IRT-DNA- sequencing.
CF=cystic fibrosis, IRT=immunoreactive trypsinogen, PAP=pancreatitis-associated protein, DNA=deoxyribonucleic acid, seq=sequencing

Laboratory techniques

The AutoDELFIA Neonatal IRT (B005-112, Perkin-Elmer, Turku, Finland) was used for determination of IRT, according to the manufacturer's protocol.
For measuring PAP, the MucoPAP ELISA (DynaBio, Marseille, France) was modified to a DELFIA method following a protocol of PerkinElmer. This is a time-resolved fluoro-immunoassay, based on a biotinylated anti-PAP-antibody and a 100 µl Eu-Streptavidin tracer. The minimal detectable value of the PAP-assay was 0.16 µg/l and the maximal value 15.8 µg/l.
DNA was extracted from dried blood spots using the EZ1 DNA tissue kit on a Biorobot EZ1 (Qiagen). Mutation analysis of the CFTR gene was performed either by screening for 35 CFTR mutations with the Line Probe Assay of Innogenetics (INNO-LiPA CFTR19 and INNO-LiPA CFTR17+Tn) or by DNA sequence analysis of all coding exons of the CFTR gene (including intron/exon boundaries) by standard procedures. Newborns with two CFTR mutations or one CFTR-mutation and one variant with unknown clinical

significance were referred. Polymorphisms and variants known to cause only male infertility were considered non disease-causing and ignored.
Sweat tests were performed by the Gibson-Cooke Quantitative Pilocarpin Iontophoresis Test (QPIT) or the Macroduct method according to international guidelines.[12]

The Dutch Paediatric Surveillance Unit

Paediatricians in the Netherlands reported all children with a new diagnosis of CF to the Dutch Paediatric Surveillance Unit (DPSU), This registration started in July 2007, and is still running. The main goal of the registration is to find infants missed by NBS.

Retrospective analysis

When parents gave permission, we performed a retrospective analysis with both screening protocols in heel prick cards of reported cystic fibrosis patients at the Dutch Paediatric Surveillance Unit as well as of patients from the four participating CF-centres born since 2003.

Statistical analysis

We determined the test characteristics (with 95% confidence intervals (CI)) of the two screening protocols (sensitivity, specificity and positive predictive value (PPV)). For determination of the sensitivity, newborns with a meconium ileus were excluded from the analysis.[13]
A power analysis was made for both specificity and sensitivity. For both strategies a cohort of 80,000 newborns would be sufficient to show that the specificity will be higher than 99.64% with a power of 80%. Assuming a sensitivity of 95% a total number of 62 CF-patients will lead to an estimated 95% CI between 85-99%. To achieve a reliable estimate of the sensitivity a cohort of known CF-patients was added to the study for a retrospective analysis.
We compared the test performances of the two strategies using a McNemar's test. P-values <0.05 were considered statistically significant.
A post-hoc analysis was done for a combined IRT-PAP-DNA-sequencing strategy using the data of both strategies. Statistical analysis was done using SPSS 17.0 software.

Results

In 2008 and 2009 145,499 newborns were screened for CF; 72,874 in 2008 and 72,625 in 2009 respectively. A total number of 372,713 newborns were born alive in the Netherlands in both years, the study region counted for 39% of all births.

IRT-PAP

Results of the IRT-PAP strategy are shown in Figure 3.2a and Tables 3.1 and 3.2. A total of 171 (0.12%) newborns were referred for a sweat test. Cystic fibrosis was confirmed in 19 newborns. One infant was missed, because of a low PAP concentration (0.8 µg/l), this infant had two mutations F508del/A455E and a positive sweat test (Chloride 65 mmol/l).

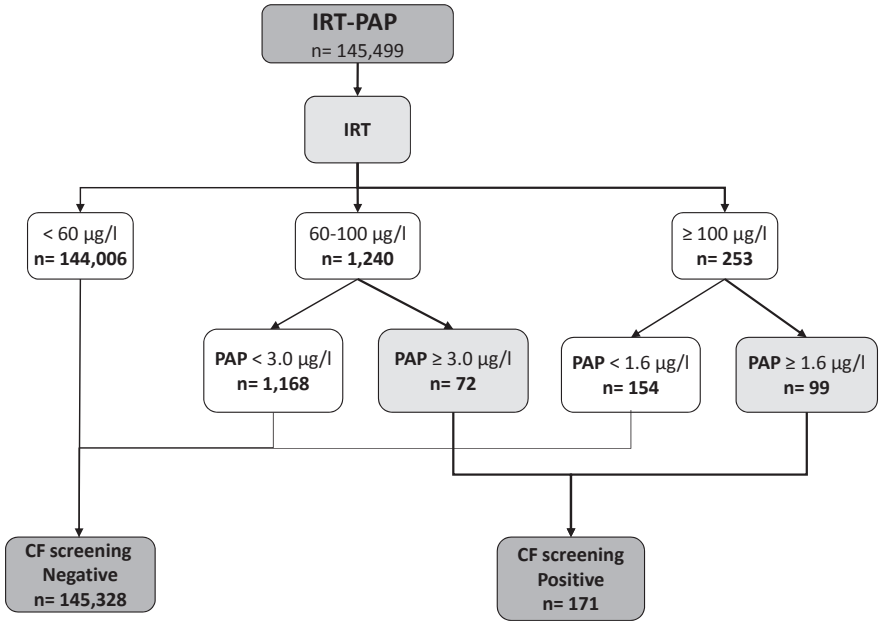

Figure 3.2a Results of the IRT-DNA-sequencing screening strategy.
Mutations included in analysis: F508del, A455E, G542X, 1717-1G>A, S1251N, R553X, R1162X, 3272-26A>G, W1282X, 2789+5G>A, 711+1G>A, E60X, N1303K, 1078delT, 3659delC, 2183 AA>G, 3905insT, R347P, 1898+1G>A, 2143delT, 2184delA, 3120+1G>A, 3199del6, 3489+10kbC>T, 394delTT, 621+1G>T, 711+5G>A, CFTRdel2,3, 1507del, G551D, G85E, Q552X, R117H, R334W, R560T, 5T, 7T, 9T. IRT=immunoreactive trypsinogen, DNA=desoxyribonucleic acid, seq=sequencing, CF=cystic fibrosis

Six infants had an equivocal sweat test result (chloride concentration 30-51 mmol/l); two of these infants were diagnosed with CF by two mutations (both F508del/F508del); one of them had an abnormal repeat sweat test (69 mmol/l). One infant had a normal repeat sweat test and no mutations. In three infants a follow-up sweat test was not performed by the paediatric pulmonologist, because the DNA

results showed no mutations. IRT and PAP concentrations for the whole population are presented in Figure 3.3.

Table 3.1 Results of three different screening strategies with an immunoreactive trypsinogen cut-off level of 60 µg/l (n=145,499).

	IRT-PAP	IRT-DNA-sequencing	IRT-PAP-DNA-sequencing
Test positive n(%)	171 (0.12)	37 (0.025)	24 (0.016)
CF with MI*	2	4	2
CF, no MI	19	20	19
False positive	146	0	0
Equivocal diagnosis†	4	13	3
Test negative	145,328	145,463	145,476
no CF	145,325	145,463	145,473
CF, with MI†	2	0	2
CF, no MI	1	0	1
Carriers	0	67	8
Sensitivity	95.0%	100%	95.0%
(95% CI)	(73.1 to 99.7)	(80.0 to 100)	(73.1 to 99.7)
Specificity	99.897%	100%	100%
(95% CI)	(99.879 to 99.912)	(99.997 to 100)	(99.997 to 100)
PPV	12.3%	64.9%	87.5%
(95% CI)	(7.9 to 8.4)	(47.4 to 79.3)	(66.5 to 96.7)

*Excluded from the analysis. †Infants with an equivocal sweat test result and/or a second mutation associated with an unclear phenotype and a normal or equivocal sweat test. IRT=immunoreactive trypsinogen, PAP=pancreatitis-associated protein, DNA=deoxyribonucleic acid, seq=sequencing CFTR gene, CF=cystic fibrosis, PPV=positive predictive value, MI=meconium ileus, CI=confidence interval

IRT-DNA-sequencing

Results are presented in Figure 3.2b and Tables 3.1 and 3.2. This strategy revealed 20 infants with CF, 13 infants with an equivocal diagnosis and 67 carriers. The referral rate was 0.026%. The PPV of this strategy was 64.9%, because infants with an equivocal diagnosis cannot be considered as having CF.

IRT-PAP and IRT-DNA-sequencing identified the same 19 patients, whereas the second strategy detected one more patient. Although the IRT-DNA-sequencing strategy was better for sensitivity, specificity, and PPV, the differences were not significant (McNemar; p=1.00). The prevalence of CF in the study region was 1:6062.

Table 3.2 Immunoreactive trypsinogen and pancreatitis-associated protein concentrations, CFTR gene mutation analysis and sweat tests in cystic fibrosis patients detected by newborn screening.

	IRT (µg/l)	PAP (µg/l)	Mutation 1	Mutation 2	Sweat test Chloride (mmol/l)
1	438	5.3	F508del	F508del	74
2	284	1.8	F508del	F508del	88
3	266	9.8	F508del	F508del	97
4	237	1.8	F508del	F508del	11 and 74
5	197	4.3	F508del	F508del	69
6*	191	12.6	F508del	C.3889dupT	94
7	164	14.4	F508del	G542X	102
8	129	4.3	F508del	F508del	failed
9	110	2.2	F508del	F508del	94
10	109	2.0	F508del	F508del	51
11	105	4.4	F508del	F508del	149
12	155	2.6	F508del	F508del	111
13	191	12.6	F508del	F508del	4
14	116	15.8	F508del	F508del	failed 3 times
15	293	5.7	F508del	2184A	120
16*	228	15.8	F508del	1294_1300del	99
17	218	4.5	F508del	G85E	99
18	153	4.0	F508del	S1251N	77
19*	141	15.8	F508del	E730X	82
20	78	0.8	F508del	A455E	65
21†	114	11.2	F508del	F508del	failed
22†	109	0.8	F508del	F508del	78
23†	93	1.3	F508del	F508del	-
24†	75	6.7	F508del	F508del	78

*Second mutation detected by sequencing, †Infants with meconium ileus (nr.21-24), those infants were excluded from the analysis. IRT=immunoreactive trypsinogen, PAP=pancreatitis-associated protein, DNA=deoxyribonucleic acid, seq=sequencing of the CFTR gene.

IRT-PAP-DNA-sequencing

A post-hoc analysis was done for a strategy consisting of a combination of both strategies: an IRT-PAP-DNA-sequencing strategy. In this strategy a DNA mutation analysis would be done in all samples with a positive IRT-PAP result, followed by sequencing when a single mutation was found. Results are presented in Figure 3.4 and Table 3.1. With this screening strategy the same 19 patients as in the IRT-PAP strategy would have been found, three infants with an equivocal diagnosis, and eight carriers.

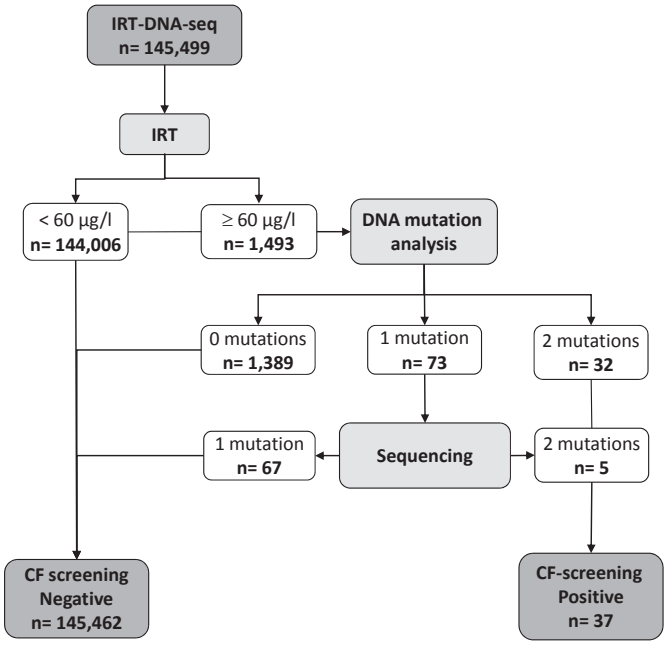

Figure 3.2b Results of the IRT-PAP screening strategy.
IRT=immunoreactive trypsinogen, PAP=pancreatitis-associated protein, CF=cystic fibrosis

Table 3.3 Immunoreactive trypsinogen and pancreatitis-associated protein concentrations, CFTR mutation analysis and sweat test results for all infants with an equivocal diagnosis.

	IRT (µg/l)	PAP (µg/l)	Mutation 1	Mutation 2	Sweat Chloride (mmol/l)
1	199	1.4	E60X	R117H-7T	36
2	139	0.8	394delTT	R117H-7T/9T	21
3	123	0.6	F508del	R117H-7T	22
4	89	1.4	S1251N	R117H-7T	29
5	79	1.6	F508del	R117H-7T	26
6	77	2.4	R553X	R117H-7T	22
7	76	0.8	F508del	R117H-7T	34
8	73	0.5	F508del	R117H-7T	25
9	70	1.0	F508del	R117H-7T	22
10	69	1.1	F508del	R117H-7T	33
11	67	2.7	F508del	R117H-7T	17
12*	174	3.8	F508del	L967S	19
13*	84	3.2	F508del	Q1352H	17

*Second mutation detected by sequencing. IRT=immunoreactive trypsinogen, PAP=pancreatitis-associated protein, DNA=deoxyribonucleic acid, Equivocal diagnosis=two CFTR mutations of which one has unclear clinical significance, and a normal or equivocal sweat test result.

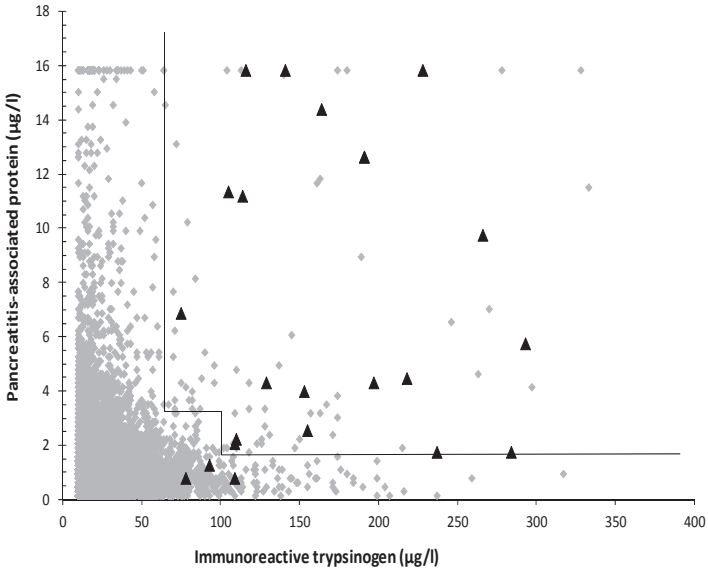

Figure 3.3 Correlation between immunoreactive trypsinogen and pancreatitis-associated protein concentrations in heel prick blood.
Cut-off levels of IRT and PAP in newborn screening for cystic fibrosis: IRT ≥60 µg/l AND PAP ≥3.2 µg/l OR IRT ≥100 µg/l AND PAP ≥1.6 µg/l. PAP=pancreatitis-associated protein, IRT=immunoreactive trypsinogen. ♦ =no cystic fibrosis, ▲ =cystic fibrosis.

Table 3.4 Time to diagnosis.

Screening strategy	IRT-PAP	IRT-DNA-seq	IRT-PAP-DNA-seq*
Median time between heel prick and screening test result, days (IQR)			
	7 (5-9)	16 (15-19)	21
Median time between screening test result and diagnosis, days (IQR)			
First year of study	4 (3-7)	4 (3-7)	4 (3-7)
Second year of study	1 (1-7)	1 (1-7)	1 (1-7)

*Predicted time period: this strategy was not tested in practice during the study. IRT=immunoreactive trypsinogen, PAP=pancreatitis-associated protein, DNA=desoxyribonucleic acid, seq=sequencing, IQR= interquartile range

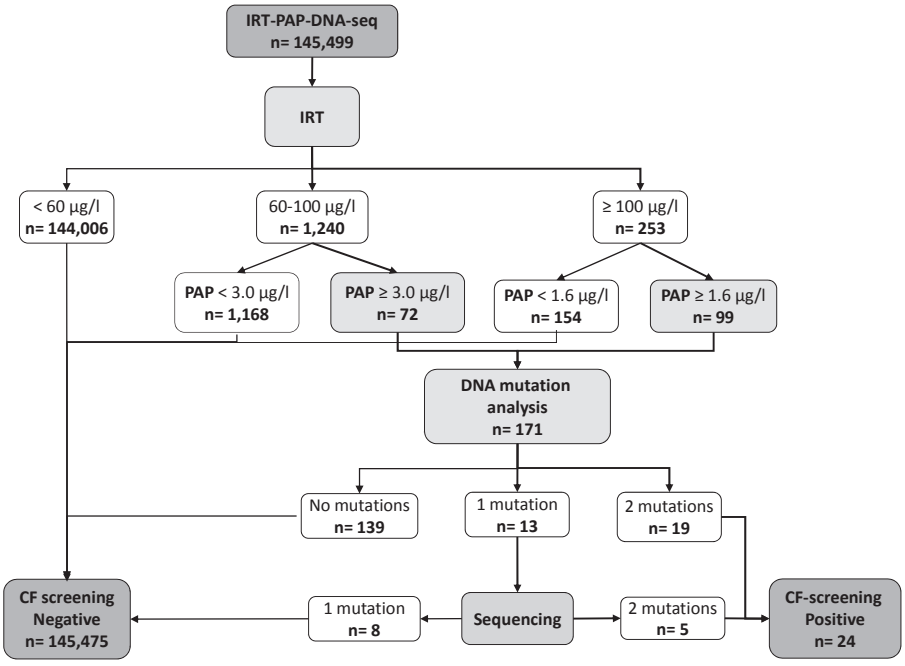

Figure 3.4 Results of the four steps of the IRT-PAP-DNA-sequencing screening strategy. IRT=immunoreactive trpysinogen, PAP=pancreatitis-associated protein, DNA=desoxyribonucleic acid, seq=sequencing, CF=cystic fibrosis.

Diagnosis

Sweat tests were performed between three and four weeks after birth. A total amount of 175 sweat tests were performed of which 145 succeeded (83%). In five infants no sweat test was done, because of a congenital lethal condition (n=3; cardiomyopathy, multiple congenital anomalies and trisomy 18), refusal of the parents (n=1; no mutations after DNA-analysis), a premature infant of 25 weeks (n=1; in this infant the DNA analysis was repeated instead of the sweat test).

The sweat test confirmed the diagnosis CF in 17 infants. The test failed in two patients, but their diagnosis could be based on two mutations (F508del/F508del), and clinical symptoms (ileum atresia and meconium plug respectively). One patient had an equivocal sweat test result, but was diagnosed because of two mutations (F508del/F508del).

The IRT-DNA-sequencing strategy revealed 13 infants with an equivocal diagnosis, all of whom had two mutations identified, one of the two being of unclear clinical significance. Three of these infants had equivocal sweat test results (chloride 33, 34, 36 mmol/l; all had R117H-7T as a second mutation), the other ten had normal sweat tests (F508del/394delTT/S1251N/R553X combined with R117H-7T n=8, F508del/L967S, F508del/Q1352H) (Table 3.3).

Time to diagnosis

Median time between heel prick and screening test result and time between screening test result and diagnosis is shown in Table 3.4. All infants with CF were diagnosed within two months after birth. In 90% of all newborns, the heel prick was taken before 144 hours after birth (day 6). Median age at heel prick was 96 hours (day 4) (Inter quartile range (IQR) 96-120). Median time between heel prick and screening test result was seven days for IRT-PAP (IQR 5-9) and 16 days for IRT-DNA-sequencing (IQR 15-19). For IRT-PAP-DNA-sequencing the predicted time between heel prick and test result is 21 days. The period of uncertainty for the parents between being informed about a positive heel prick result and confirmation or exclusion of the diagnosis was 4 days (IQR 3 to 7) in the first year of the study, and one day (IQR 1 to 7) in the second year, for both strategies.

DPSU and the retrospective analysis

Between January 2008 and April 2011, no false-negative infants were reported.
A retrospective analysis was performed in 74 heel prick cards. IRT and PAP concentrations declined with age of the heel prick card, therefore the results of the retrospective analysis could not be used to determine the sensitivity of this strategy. Our mutation panel detected two mutations in 66 cards, one mutation in seven cards and no mutations in one card. Eight cards were sequenced and after sequencing all cards contained two mutations. The 35-mutation panel therefore had a sensitivity of 98.6% (95% CI 91.6 to 99.9) for detection of one or two mutations.

Discussion

As far as we know, our study is the first prospective study comparing two novel screening strategies in NBS for CF (IRT-PAP and IRT-DNA-sequencing). The best test performance was found for IRT-DNA-sequencing. A post hoc analysis of a combination of both strategies (IRT-PAP-DNA-sequencing) resulted in a program with a sensitivity similar to IRT-PAP but a higher specificity and PPV. This third strategy led to detection

of considerably fewer carriers and fewer equivocal diagnoses than current screening strategies. This strategy has never been studied before.

All CF patients were diagnosed within two months after birth, which is within the window of opportunity to create a better prognosis.[14]

Our study has some limitations that should be considered in the interpretation of the results. The currently most applied screening strategy for NBS for CF is the IRT-DNA strategy with referral of all infants with one or two mutations. In an ideal study design this strategy would have been used as the "gold standard". Using our study data an IRT-DNA program would have had a sensitivity of 100% (88.2 to 100), a specificity of 99.954% (99.941 to 99.964) and a PPV of 26.4% (17.9 to 36.8), this may be investigated prospectively in a new study comparing those protocols. However, sequencing the *CFTR* gene in all infants with a single mutation and an equivocal sweat test is advised as the optimal diagnostic strategy in the current European consensus guidelines,[11] and this most probably has a similar sensitivity as performing a sweat test in infants with a single CFTR-mutation. Moreover, within our study it was possible to compare specificity and PPV with the "gold standard" strategy, the IRT-DNA-sequencing strategy appears to have a considerably better specificity as well as PPV. We did not find a statistical significant difference betweenthe IRT-PAP and IRT-DNA-sequencing strategy, this may have been caused by the relatively small number of CF patients in our screened population, which is also a limitation of our study.

The diagnosis was confirmed by the sweat test in 17 of the 20 infants. In all cases the diagnosis could be confirmed according to the international definition,[10,11] by either a sweat test or a second DNA analysis and clinical symptoms and/or a sibling with CF. We used the Dutch Paediatric Surveillance Unit (DPSU) to detect children with CF who were potentially missed by screening.

The power analysis showed that we needed 62 patients to determine the sensitivity reliably. In the study design a retrospective analysis of known patients was planned to calculate the sensitivity, but this partly failed because IRT and PAP concentrations decreased over time (results not shown). The prevalence of CF in our study was 1:6062. In 2005 and 2006, the Dutch CF-registry registered a nationwide prevalence of 1:5000 comparable to the prevalence of 1:4750 published in 2005.[15] The reason for this difference may be that the prevalence of CF is declining as it is in whole Europe.[16,17] Causes for this decline may be preconception and prenatal screening or parents deciding not to have any other children and NBS with detection of carrier-couples. Another cause may be a lower prevalence of CF in the southern part of the Netherlands where our study was performed.[18,19]

Our results for IRT-PAP are comparable to the findings of two previous studies.[6,7] A previous study found a sensitivity of 100% for detection of CF with a specificity of 99.81% and a PPV of 9.4%.6 A prospective study comparing IRT-PAP with IRT-DNA

showed a sensitivity of 85.7%, a specificity of 99.90%, and a PPV of 12.2% for the IRT-PAP strategy.[7] The sensitivity in the last study appears low, but this study used a slightly different protocol.

Internationally used screening programs consisting of IRT, IRT-IRT, or IRT-DNA (one or more mutations) show variable test performances.[4] IRT-IRT programs show a sensitivity between 80.2% and 96.8%, with a specificity of 99.8%.[20,21] In DNA-based programs (IRT-DNA, IRT-DNA-IRT, IRT-IRT-DNA), the sensitivity varies depending on the mutation panel, the IRT cut-off level and the fail-safe procedures. Previous studies showed sensitivities of 96.0 to 99.5% with specificities between 99.60% and 99.97%.[22-24]

IRT-PAP has advantages compared to DNA-based programs: First, no carriers are detected. Although carrier detection is sometimes considered as an advantage, this is not a universal opinion.[25-28] The advantages of detection of trait-trait couples and extended family screening do not counterbalance this disadvantage in our opinion. Secondly, no second heel prick is needed in contrast to IRT-IRT programs. Disadvantages are the high number of false-positive test results, and a long period of uncertainty and parental stress due to the frequent failure of the sweat test.

The IRT-DNA-sequencing strategy had the best test performance in our study, but this strategy led to equivocal diagnoses and identified carriers. However, the number of referrals was considerably lower and the specificity and PPV higher than those of the currently most applied screening strategy, IRT-DNA. In contrast to current IRT-DNA based programs the advantage of the IRT-DNA-sequencing approach was that parents were not aroused by a positive screening-test result when a single mutation was identified.[29] The information leaflet about NBS for CF that the parents received at three occasions (during pregnancy, when registering the baby and immediately before the heel prick) mentioned that parents could ask for the DNA results, but very few parents did so (0.007%).

Eleven of the 13 infants with an equivocal diagnosis in the IRT-DNA-sequencing strategy had R117H-7T as second mutation. Most of them had normal sweat test results (Table 3.3), this means that they will probably have a normal or subnormal CFTR function. The Dutch CF-registry showed only 10 patients (1196 registered patients in 2008) with a R117H-7T mutation, and only four of them were diagnosed under the age of 18 years. Our findings confirm an earlier observed discrepancy in frequency of this mutation in screened populations and CF-registries.[30,31] This indicates that this mutation mostly acts as a non-disease-causing variant. Many NBS for CF experts therefore advise exclusion of this mutation.[31] If R117H-7T would be excluded from the panel, only two infants with an equivocal diagnosis would have been identified with this strategy. With a IRT-DNA strategy these two infants were probably not identified as having an equivocal diagnosis, but only as carriers.

Because of the multi-ethnic Dutch population, infants with two rare mutations in the non-Caucasian population might be missed when using a DNA-based screening strategy. But in the retrospective analysis we identified only one patient that would not have been detected by the IRT-DNA-sequencing analysis. The IRT-PAP strategy reveals CF patients because of high IRT and PAP levels, which makes this a more robust screening strategy when the screened population has ethnic differences.

Conclusion

All three studied screening strategies seem useful for NBS for CF, but the choice which strategy to implement depends on the requirements of the test. Both strategies (IRT-PAP and IRT-DNA-sequencing) performed well, better than expected, and although there was no statistical significant difference the IRT-DNA-sequencing strategy detected one infant that was missed by IRT-PAP. This strategy also leads to less referrals and therefore to a higher specificity and PPV than the current IRT-DNA strategy. IRT-PAP may be the optimal choice if the use of DNA-technology must be avoided. When identification of carriers or false-positive results are considered to be important disadvantages and the number of equivocal diagnosis should be minimised IRT-PAP-DNA-sequencing may be the best choice. The Dutch ministry of health decided to implement this last strategy in the Dutch NBS programme as of May 1st 2011.[32]

Acknowledgements

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CHAPTER 4

The influence of sex, gestational age, birth weight, blood transfusion, and timing of the heel prick on the pancreatitis-associated protein concentration in newborn screening for cystic fibrosis

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Abstract

Background

Pancreatitis-associated protein (PAP) is currently discussed as a marker in newborn screening (NBS) for cystic fibrosis (CF). However, it is not known if the PAP concentration is influenced by sex, gestational age, birth weight, blood transfusion or time of collection.

Methods

In 2008, all newborns in part of the Netherlands were screened for CF by an IRT-PAP protocol. PAP concentration was determined by the MucoPAP ELISA (DynaBio), which was modified to a dissociation enhanced lanthanide fluoroimmunoassay (DELFIA) method following a protocol of PerkinElmer.

Results

72,874 newborns were screened. In healthy newborns, the median PAP concentration was 0.5 µg/l (Interquartile range (IQR) 0.3-0.8) whereas this was 3.2 µg/l (IQR 2.0-12.5) in CF infants. PAP concentrations were lower in premature infants 0.94 and 0.91 times for 25 to 31+6 weeks GA and 32 to 36+6 weeks respectively. A higher PAP concentration was observed in low-birth-weight infants (<2500 gram)(p=0.001), per 100 gram birth weight gained the PAP concentration decreased with 0.1%. PAP levels were higher after a blood transfusion, the 95th percentile increased from 1.3 to 3.6 µg/l leading to a higher false-positive rate. The PAP concentration increased when newborn screening was performed more than 168 hours (day 7) after birth (β=1.63), the 95th percentile increased from 1.3-1.6 µg/l to 4.0 µg/l after 168 hours.

Conclusion

Sex, birth weight, and gestational age lead to small differences in PAP concentrations without consequences for the screening algorithm. However, blood transfusion as well as performance of the heel prick after 168 hours (7 days) lead to clinically significant higher PAP levels and to a higher risk on a false-positive screening test result.

Introduction

Newborn screening (NBS) for cystic fibrosis (CF) is implemented in an increasing number of countries worldwide.[1] Current screening protocols all start with measurement of immunoreactive trypsinogen (IRT) followed by a CFTR-mutation analysis of one or more mutations, or a second IRT test after 2-4 weeks.[2] A major drawback of screening protocols using CFTR-mutation analysis is the high number of identified healthy carriers and cases with an equivocal diagnosis.

Pancreatitis-associated protein (PAP) is a 16 kD secretory protein.[3] It was identified in 1984 in rats as a protein secreted by the pancreas after induction of pancreatitis.[4] PAP serum levels may be used as a biological marker of pancreatitis.[5,6] It is thought that PAP limits systemic complications in acute pancreatitis by reducing leucocytes-induced lung-injury.[7] PAP is not produced in a healthy pancreas but synthesized in high amounts after pancreatic stress, not only after pancreatitis but also in systemic infections, after hypoxic events, and after abdominal surgery.[3,7,8]

Three studies showed high PAP blood concentrations in neonates with CF.[6,9,10] The combination of both elevated levels of IRT and PAP were a stronger indicator for CF than IRT alone. In the Netherlands we performed a prospective study on novel strategies in 2008 and 2009; an IRT-PAP strategy was compared to an IRT-DNA-sequencing strategy.[11] In this study we evaluated the potential effects of sex, gestational age, birth weight, blood transfusion and time of heel prick on blood concentrations of PAP in a newborn population in the Netherlands.

Methods

Study population

All newborns from four out of 12 provinces in the middle and southeast of the Netherlands born in 2008 were included in the study, unless the parents refused the screening for CF. Heel prick samples were collected on filter paper in term and preterm babies between 72 and 168 hours after birth. Information on sex, gestational age, birth weight, time of heel prick and blood transfusion was recorded on the heel prick card.

Screening protocol

This study was part of a large study in the Netherlands assessing the test characteristics of two new algorithms for CF screening (CHOPIN study).[11] The IRT-PAP protocol consisted of measurement of the concentrations of IRT and PAP in all samples. A positive result was defined as a combination of either IRT ≥100 µg/l and PAP ≥1.6 µg/l, or IRT ≥50 µg/l and PAP ≥3.0 µg/l, as described before[6,9] and corrected

according to the publication at www.isns-neoscreening.org/htm/news in March 2011. The diagnosis CF was confirmed or excluded by a sweat test performed according to international guidelines by two methods; the Gibson-Cooke Quantitative Pilocarpin Iontophoresis test (QPIT) or Macroduct method.[12,13]

Definitions

A diagnosis of CF was confirmed by a sweat chloride concentration of ≥60 mmol/l and/or a meconium ileus or positive family history.[14,15]
An equivocal diagnosis was defined according to international standards as an equivocal sweat test result (chloride 30-60 mmol/l) or a normal sweat test result (chloride <30 mmol/l) on two occasions in a newborn with two CFTR mutations of which one or both have unclear clinical consequences (DNA results were known because of the IRT-DNA-sequencing strategy).[15,16] In the IRT-PAP strategy CF was excluded when the chloride concentration was below 30 mmol/l.

Laboratory techniques

The AutoDELFIA Neonatal IRT (B005-112, PerkinElmer, Turku, Finland, www.perkinelmer.com) was used for determination of IRT, according to the manufacturer's protocol. For measuring PAP the MucoPAP enzyme-linked immunosorbent assay (ELISA) (DynaBio, Marseille, France, www.dynabio.com) was modified to a time-resolved dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA) method following a protocol of PerkinElmer. Filter paper disks (3 mm) containing 3 µl blood, were extracted with 150 µl elution buffer (Phosphate-Buffered Saline (PBS)) in wells of a microtiterplate. After 10 minutes shaking on a DELFIA Plateshake, the plates were incubated in a refrigerator (4°C) for 16 hours. One hundred µl of the extracts was transferred to an anti-PAP coated plate, together with 100 µl of control samples and PAP calibrators. After incubation at room temperature for 3 hours while slowly shaking, the plates were washed. Then 100 µl of biotinylated anti-PAP-antibody solution was added. The plate was covered and shaken for 30 minutes. After this incubation step and washing of the plates, 100 µl Eu-Streptavidin solution (tracer) was added. The plates were covered, shaken for 30 minutes and washed. Enhancement solution (200 µl) was added and plates were shaken slowly for 5 minutes. Fluorescence was measured immediately after that in the time-resolved fluorometer. The minimal detectable value of the assay was 0.1 µg/l and the maximal value 9.9 µg/l. All values higher than 9.9 µg/l were therefore recorded as 9.9 µg/l.
In March 2011 the ISNS published a change in dilution factor because a 3mm punch contains 3 µl blood instead of 5 µl which changes the dilution factor to 150/3 instead of 150/5 (www.isns-neoscreening.org/htm/news). This changed the concentrations for

PAP (1.6 times higher) but not the test performance. In this study we used the correct concentrations.

Statistical analysis

Statistical analysis was done using SPSS 17.0 software. All screened newborns were included in the analysis. Non-parametric tests were used for determination of differences between groups; the Mann-Whitney-U test was used for the differences between categories.
A multiple linear regression analysis was done to determine the influence of sex, gestational age, birth weight, blood transfusion and age at the heel prick on the PAP concentration. We used logistic regression to determine the risk on a PAP concentration above 1.6 and 3.0 µg/l. P-values <0.05 were considered statistically significant. To test the robustness of the linear regression model we used truncation and stratification.

Results

Pancreatitis-associated protein

The intra assay and inter assay variation for samples around the cut-off levels varied between 10 and 15%. The PAP concentration could be determined in all samples. The majority of infants (94.7%) had PAP concentrations below 1.6 µg/l, 4.1% had concentrations between 1.6 and 3.0 µg/l, and 1.2% had a PAP concentration of 3.0 µg/l or higher (Figure 4.1). PAP concentrations in CF patients were significantly higher than in screen-negative infants (Mann-Whitney-U; $p<0.001$), as shown in Figure 4.2. The median PAP concentration in newborns with CF without meconium ileus (n=10) was 3.2 µg/l (IQR 2.0-12.5) versus 0.5 µg/l (IQR 0.3-0.8) in screen-negative infants (n=72,754)($p<0.001$). One screen-negative infant with CF and a meconium-ileus had a PAP-concentration of 0.8 µg/l. Carriers and infants with an equivocal diagnosis had median PAP concentrations of 0.8 (IQR 0.6-1.3) and 1.4 (IQR 0.6-2.4) respectively.

Statistical analysis of multiple variables on the PAP concentration

Median PAP concentration and Odds ratios for PAP concentrations above 1.6 and above 3.0 for different infant categories are shown in Table 4.1. Results of the multiple regression analysis are shown in Table 4.2, the results of the analysis did not differ significantly after truncation at 1% and 99%.
The influence of each specific variable is corrected for other variables by including these simultaneously into a regression model. Birth weight was included as a continuous variable, while sex, GA, blood transfusion and time of heel prick were

included as categorical variables. Sex and blood transfusion were included as dummies (male/female and yes/no respectively), GA was split into three categories, of which the highest age was the reference group and age of heel prick was split in three categories in which 72-168 hours was reference the category. Since the residual errors of this model were not normally distributed, but highly skewed to the right, the PAP concentration was natural log transformed and subsequently, regressed on sex, GA, birth weight, blood transfusion and age at heel prick. This model yielded normally distributed residual errors, and as a result, multiple linear regression with a natural log transformed PAP concentration was appropriate.

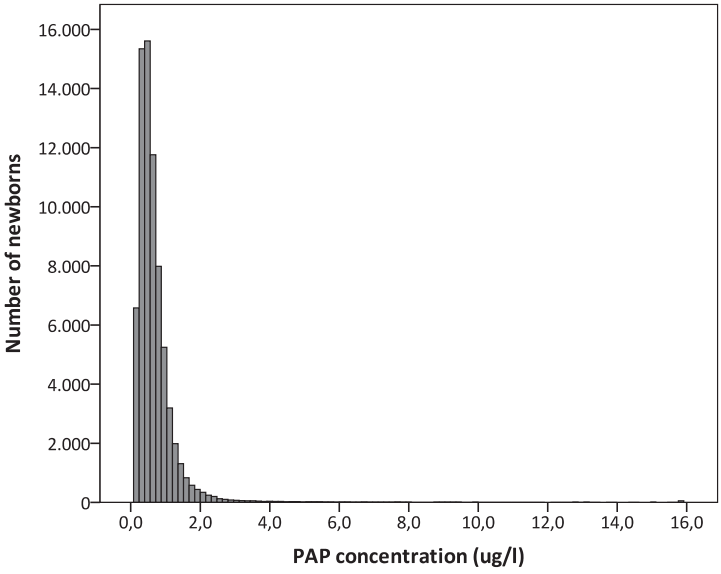


Figure 4.1 Distribution of PAP concentrations 72,874 newborns screened for CF.
Distribution of the concentration of PAP in a newborn population of 72,874 infants in the Netherlands. Columns represent the number of newborns with a certain PAP concentration. The minimum measurable concentration was 0.16 µg/l, the maximum 15.8 µg/l. PAP=pancreatitis-associated protein, CF=cystic fibrosis.

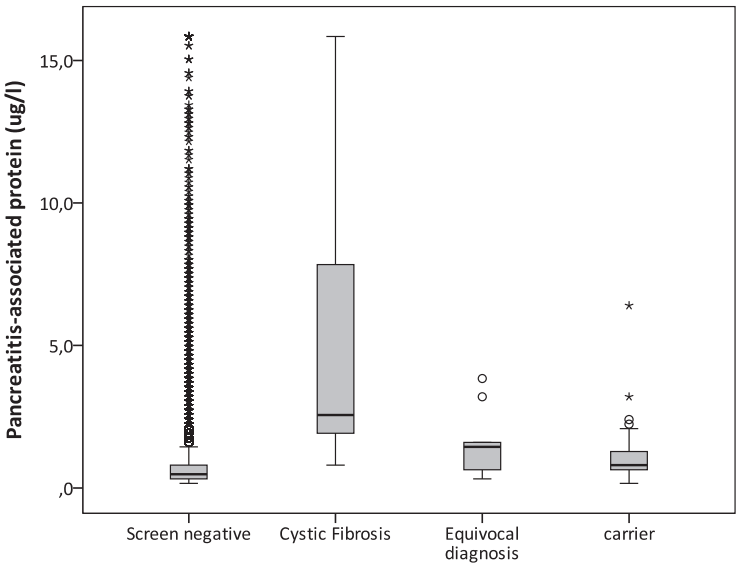


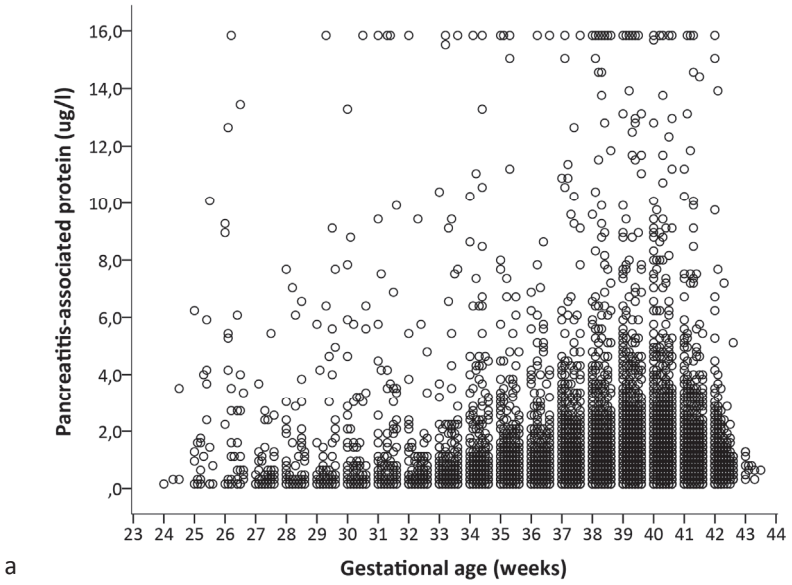
Figure 4.2 Concentrations of pancreatitis-associated protein in CF infants, equivocal diagnosis, carriers and screen-negative infants.
PAP concentrations µg/l in newborns with a negative screening test result for CF, newborns with classic CF. Median values are indicated with a flat line, the blocks show the IQR and 1.5 IQR from the median concentration is indicated with a line. PAP=pancreatitis-associated protein, CF=cystic fibrosis, IQR=interquartile range

Sex

Males showed lower PAP concentrations than females, the β was 0.90 (95% CI 0.89 to 1.12) This difference is not significant and the 95th percentile is comparable, 1.3 and 1.4 µg/l respectively. Odds ratio's for PAP levels above 1.6 and 3.0 µg/l were 0.66 and 1.0 respectively, so sex does not increase the risk on a positive screening test result.

Birth weight

A higher birth weight was significantly associated with a lower PAP concentration (p=0.002), even when adjusted for gestational age. Figure 4.3 shows PAP levels for birth weight in the screened population. For each 100 gram in birth weight gained, the PAP concentration decreased with 0.1%. The odds ratio for the risk of a PAP concentration above 1.6 µg/l was 0.98, and for the cut-off level of 3.0 µg/l this was 0.96 per 100 gram weight gained. The specificity of IRT-PAP for low birth weight infants (<2500 gram) was 99.6% (95% CI 99.3-99.8), which increased to 99.9% for normal-birth-weight infants (≥2500 gram).



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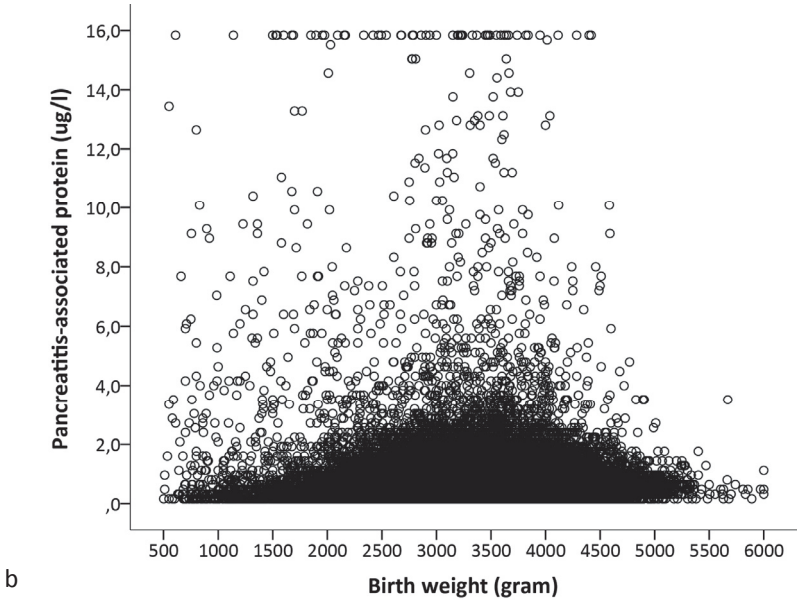

b

Figure 4.3 Distribution of pancreatitis-associated protein concentrations (n=72,874) for gestational age (a) and birth weight (b).

Gestational age

Figure 4.3 shows PAP concentrations for GA in the screened population. Compared to the reference category of term infants (>37 weeks GA), prematurity was significantly associated with a lower PAP concentration, with a β of 0.94 (95% CI 0.92 to 0.96) for a GA of 32 to 36+6 and 0.92 (95% CI 0.86 to 0.97) before 32 weeks GA. Premature infants have a higher risk on a PAP concentration of 1.6 µg/l or higher (OR 1.70 for 25-31+6 weeks and 1.07 for 32-36+6 weeks) and for concentrations above 3.0 µg/l the OR was 1.81 between 25 and 32 weeks and 3.38 between 32 and 37 weeks GA. The specificity of IRT-PAP for premature babies (<32 weeks GA) was 99.4% (95% CI 98.4-99.8), which increased to 99.8% after 37 weeks (95% CI 99.8-99.9).

Blood transfusion

Blood transfusion led to increased PAP concentrations with a factor 1.07% (95% CI 0.97 to 1.18). The 95th percentile was 3.6 µg/l after a blood transfusion compared to 1.3 µg/l in the non-transfused population. The odd ratio for a PAP levels of 1.6 µg/l or higher was 1.89 and 0.92 for PAP levels above 3.0 µg/l (Table 4.1). An IRT-PAP screening program would have a specificity of 97.4% after a blood transfusion were this would be 99.9 in the non-transfused population.

Age at heel prick

About 99% of all heel prick samples were taken between 72 and 168 hours after birth. A heel prick sample taken before 72 was a little different from a sample taken between 72 and 168 hours after birth (β 1.11 95% CI 0.85-1.45), but both PAP concentrations were significantly lower than after 168 hours of age, with a β of 1.64 (95% CI 1.57 to 1.71) The nineteen infants screened before 72 hours (on day 2 after birth) had PAP concentrations between 0.16 and 1.28 µg/l. All infants except one (36+2 weeks GA) were born at term (38 to 41+4 weeks), birth weight between 2140 (premature infant) and 4695 gram, and none received a blood transfusion. The odds ratio for a PAP concentration above 1.6 µg/l was 8.54 when the heel prick was performed after 168 hours after birth, and 14.22 for the risk on a PAP concentration above 3.0 µg/l (Table 4.1).

Table 4.1 Group characteristics and number of infants and Odds ratio for a PAP concentration above 1.6 and 3.0 µg/l (cut-off levels) and consequences for the specificity of an IRT-PAP screening program (with IRT cut-off level 50 µg/l).

Characteristic	Infants, n (%)	Median PAP µg/l (95^{th} percentile)	PAP ≥1.6 µg/l n (%)	OR PAP ≥1.6 µg/l (95% CI)	PAP ≥3.0 µg/l n (%)	OR PAP ≥3.0 µg/l (95% CI)	Specificity IRT-PAP % (95% CI)
Sex	70,010 (96.1)		3655		827		
Male	35,841 (51.2)	0.5 (1.3)	1472 (4.1)	0.66 (0.59-0.72)	408 (1.1)	1.0 (0.82-1.2)	99.8 (99.8-99.9)
Female	34,169 (48.8)	0.6 (1.4)	822 (2.4)		414 (1.2)		99.8 (99.8-99.9)
Birth weight	70,091 (96.2)		3665	per 100 gram 0.98 (0.97-0.99)	826	per 100 gram 0.96 (0.94-0.98)	
<2500 gram	4,077 (5.8)	0.5 (1.4)	435 (10.7)		190 (4.7)		99.6 (99.3-99.7)
≥2500 gram	66,009 (94.2)	0.5 (1.3)	3230 (4.9)		636 (1.0)		99.8 (99.8-99.9)
Gestational age	70,081 (96.2)		3665		828		
<32 weeks	691 (1.0)	0.3 (2.8)	118 (17.1)	1.70 (1.14-2.54)	72 (10.4)	3.38 (1.79-6.39)	99.4 (99.4-99.8)
32-37 weeks	4,713 (6.7)	0.5 (1.3)	345 (7.3)	1.07 (0.87-1.31)	133 (2.8)	1.81 (1.27-2.57)	99.7 (99.4-99.8)
≥37 weeks	64,677 (92.3)	0.5 (1.3)	3202 (5.0)	0*	623 (1.0)	0*	99.8 (99.8-99.9)
Blood transfusion	67,209 (92.2)		3517		785		
No	67,018 (99.7)	0.5 (1.3)	3460 (5.1)	0*	757 (1.1)	0*	99.9 (99.8-99.9)
Yes	191 (0.3)	0.6 (3.6)	57 (29.9)	1.89 (1.11-3.21)	28 (14.7)	0.92 (0.41-2.10)	97.4 (93.6-99.0)
Age at heelprick	69,036 (94.7)		1822		416		
<72 hours	19 (0.03)	0.5 (1.6)	1 (5.3)	2,42 (0.32-18.26)	0	n/a	100 (79.1-100)
72-168 hours	68,249 (98.8)	0.5 (1.3)	1669 (2.4)	0*	350 (0.5)	0*	99.9 (99.9-99.9)
>168 hours	768 (1.1)	0.5 (4.0)	152 (18.8)	8.54 (6.95-10.48)	66 (7.7)	14.23 (10.38-19.50)	99.1 (98.0-99.6)

*reference category. PAP=pancreatitis-associated protein, IRT=immunoreactive trypsinogen, OR=odds ratio, n/a=not applicable.

Table 4.2 General linear regression model for dependent variable LnPAP, data expressed are inverse Ln (=e^x). This model shows the influence of sex, gestational age, birth weight, blood transfusion and time of heel prick.

	ß (95% CI)	p	After truncation <1% and >99%
Sex			
Male	0.82 (0.81 to 0.83)	<0.001	0.82 (0.82-0.82)
Female	0*		
Gestational age			
25-31+6 weeks	0.58 (0.55 to 0.61)	<0.001	0.53 (0.50-0.56)
32-36+6 weeks	0.80 (0.78 to 0.81)	<0.001	0.78 (0.77-0.78)
≥37 weeks	0*		
Birth weight			
(per 100 gram)	1.00 (1.0-1.0)	0.002	1.0 (1.0-1.0)
Blood transfusion			
Yes	1.07 (0.97-1.18)	0.170	1.02 (0.92-1.12)
No	0*		
Time of heel prick			
<72 hours	1.11 (0.85-1.45)	0.437	1.12 (0.87-1.45)
72-168 hours	0*		
≥168 hours	1.64 (1.57-1.71)	<0.001	1.46 (1.39-1.52)

*This category is reference category
ß =beta, CI=confidence interval

Discussion

Sex, gestational age, and birth weight influenced PAP-concentrations but not substantially affected the screening protocol, while blood transfusion and time of heel prick after 168 hours led to higher PAP levels and therefore to a higher false positive rate.

A few results were especially interesting and have not been described earlier.

First, premature infants (GA <32 weeks) had a lower PAP concentration than term infants. However, the chance on a PAP level above the cut-off level was higher in this group and the specificity of an IRT-PAP screening program would be lower for premature infants. Probably, the lower PAP concentration in premature infants may be explained by a lower functional base-level, because of immaturity of the pancreas. This may be caused by perinatal stress, hypoxic events, gastro-intestinal problems, infections or congenital abnormalities. It is known that these factors may cause or follow after premature birth, and stress will make the pancreas produce PAP.[6] The lower PAP concentration did not lead to false negative screening results, as so far no

false negative infants (screened in 2008) have been reported. Further studies are needed to elucidate more precisely the relation between prematurity, clinical background and PAP concentration

Secondly, PAP concentrations were higher in the majority of low-birth-weight infants (<2500 gram), even when adjusted for GA. This finding seems in discrepancy with the observation of lower PAP concentrations in premature infants. PAP concentrations seem to be normally distributed for birth weight as shown in Figure 4.3. In some low-birth-weight infants PAP levels are very high, leading to false-positive screening results. This may be caused by smaller numbers in the lower ranges or the relatively high number of infants with perinatal problems causing low birth weight, such as chromosomal abnormalities, intrauterine infection, maternal hypertension, or placental insufficiency. Perinatal problems are associated with foetal stress, and stress causes PAP production in the pancreas. Our hypothesis is that growth retardation may be caused by longstanding intrauterine stress, therefore higher PAP levels may be found in low-birth-weight mature infants. Further study is needed to investigate other causes of high PAP levels in low-birth-weight infants.

Thirdly, transfusion with adult blood resulted in significantly higher concentrations of circulating PAP in some newborns, presumably because of the much higher levels of PAP found in adult blood.[17] We advise to repeat the heel prick after a certain time as is agreed on for other diseases in NBS.[18,19] The optimal time for a repeat test is not known and has to be determined. We found no evidence for a higher risk of false-negative results after blood transfusion, IRT concentrations were above 50 µg/l in 7.3% of the screened newborns having received a transfusion compared to 2.5% in the non-transfused population (results not shown).[11]

Fourthly, we observed that PAP concentrations were higher after day 7, counting the day of birth as day 0, leading to a lower specificity than when performed between 72 and 168 hours. When newborn screening was performed before 72 hours after birth, the PAP concentration was found to be a little higher, however the odds ratio for PAP levels above the cut-off of 1.6 or 3.0 µg/l were not significantly different.

Finally, it is known that infants with a meconium ileus may have low IRT concentrations, which was not the case in the child with meconium ileus in our study (IRT 109 µg/l).[20] It has been shown previously that PAP concentrations may be low in newborns with meconium ileus as well.[6] In our patient the first heel prick showed a low PAP concentration (0.8 µg/l), a second heel prick was done after surgery and showed an elevation of PAP to 10.0 µg/l, but this may also be caused by stress associated with abdominal surgery.

Conclusion

Sex, gestational age, and birth weight lead to small differences in PAP concentrations but these differences do not lead to a recommendation to adjust cut-off levels for certain groups in NBS for CF. However, blood transfusion, and tests performed after 168 hours (7 days after birth) as well as low birth weight may lead to significantly higher PAP concentrations, and to a higher false-positive rate. We recommend a repeat blood sample after a blood transfusion and to strict compliance of the recommended day of the heel prick.

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CHAPTER 5

Cost-effectiveness of three novel strategies for newborn screening for cystic fibrosis

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Abstract

Background

Earlier cost-effectiveness studies showed that newborn screening for CF (NBSCF) is a good economic option with positive health effects and longer survival being expected. Since then, pancreatitis-associated protein (PAP)-testing was described as a new promising second tier in NBSCF.

Aim of the study

To assess the cost-effectiveness of two new screening strategies, IRT-PAP and IRT-DNA- sequencing

Methods

We used a previously developed decision analysis model to compare costs and effects of NBSCF with a situation without screening. The model parameters were based on the results of the primary data collected in the screening study performed in 2008 and 2009 in which 145,499 newborns participated, and extrapolated to a cohort of 185,000 neonates, the approximate annual number of births in the Netherlands. For some parameters literature review and expert opinions were used. A post-hoc analysis was performed for the IRT-PAP-DNA-sequencing and IRT-DNA strategies using the same data.

Results

Compared to a situation without screening, screening strategies had cost-effectiveness ratios (CER) varying from €23,600 to €29,200 per life-year gained. The IRT-PAP strategy had the most favourable cost-effectiveness ratio. Additional life-years can be gained by the IRT-DNA strategy but against higher costs. In a situation of 5% reduction in treatment costs, screening even leads to financial savings.

Conclusion

Newborn screening for CF is economically justified. IRT-PAP is the best economic option. All screening strategies may result in cost savings when early diagnosis and treatment leads to a 5% reduction in life time costs of treatment.

Introduction

Cystic fibrosis (CF) is one of the most common autosomal recessively inherited disorders in Caucasian populations. Early detection of patients with CF by newborn screening aims to start treatment as early as possible to prevent malnutrition and irreversible lung damage, and to improve life expectancy. Early diagnosis of CF also enables timely genetic counselling of the parents on the risk of CF in a following pregnancy.

Cost-effectiveness of newborn screening for CF (NBSCF) was assessed on a hypothetical birth cohort of 200,000 newborns for different screening strategies based on immunoreactive trypsinogen (IRT) and DNA tests.[1] It showed that NBSCF is a good economic option, with positive health effects being expected. Pancreatitis-associated protein (PAP)-testing was described as a new promising second tier in NBSCF.[2,3] The CHOPIN study (Cystic fibrosis Heel prick amOng a newborn Population In the Netherlands) evaluated two novel strategies for NBSCF, IRT-PAP and IRT-DNA-sequencing.[4] The aim of this study was to assess the cost-effectiveness of novel screening strategies with use of primary data derived from the CHOPIN study and the previously developed economic model.[1]

Materials and methods

We used a decision analysis model to compare costs and effects of neonatal screening for CF with a situation without screening in the Netherlands.[1] The model parameters were based on the results of the primary data collected in the CHOPIN study,[4] and extrapolated to a cohort of 185,000 neonates, the approximate annual number of births in the Netherlands.[5] For some parameters literature review and expert opinions were used. We used the same decision analysis model as in the earlier study and refer for details to this study.[1]

All of the costs and effects were discounted at a rate of 3% to convert future costs and effects to their present value, as recommended by the Panel on Cost-Effectiveness in Health and Medicine.[6] The costs and consequences of each strategy were assessed from a societal perspective, which means that all costs and consequences are incorporated regardless of who incurs the costs and who obtains the effects. Costs are in 2009 Euros (€).

Epidemiology

The birth prevalence of CF in the Netherlands is estimated to be 1 in 4,750.[7] Within an annual birth cohort of 185,000 newborns approximately 39 children with CF are born

each year. Meconium ileus (MI) is the presenting sign in about 17% of newborns with CF.[8] We assumed a life expectancy of 40 years.[9-11]

Newborn screening

The percentage of children participating in NBS in the Netherlands was 99.8% in 2008-2009.[12,13] In the CHOPIN study, only 0.09% refused CF screening (personal communication L.H. Elvers). Figure 5.1 shows the four strategies that we studied.
First, an IRT-test followed by a PAP-test if positive (IRT-PAP), 2) IRT followed by DNA mutation analysis of 35 frequently occurring CFTR mutations (DNA), followed by sequence analysis of the entire coding region of the CFTR-gene of all samples with only one CFTR mutation (IRT-DNA-seq). In a post-hoc analysis, we evaluated two extra strategies. As a third strategy, a combination of both strategies was evaluated (IRT-PAP-DNA-seq): IRT if positive followed by PAP, DNA analysis when PAP was above cut-off, and sequencing if DNA analysis revealed one CFTR mutation. Screening tests were only positive when two CFTR mutations were identified. As fourth strategy we evaluated IRT-DNA: IRT followed by DNA if IRT is above cut-off point. The test is positive when one or two pathogenic CFTR mutations are identified.[6,14,15] Data on sweat tests were lacking in the screen negative babies with one mutation with DNA and no mutations after sequencing. We considered these cases as healthy carriers, and assumed a normal sweat test.
Screen-positive newborns were referred for a sweat test to confirm or exclude CF. The diagnosis CF was confirmed when the chloride concentration was ≥60 mmol/l in combination with two mutations or symptoms. An equivocal sweat test (chloride 30-60 mmol/l) was repeated in the IRT-(PAP)-DNA-seq strategy. In the IRT-PAP strategy, an abnormal or equivocal sweat test led to DNA analysis, and when only one mutation was found to sequencing. A similar procedure was applied in the IRT-DNA strategy.

Specificity and sensitivity

Specificity and sensitivity were determined for each test separately, as the number of tests needed in step 2 to 5 (Figure 5.1) depends on the specificity and sensitivity of the previous tests.
Our population study was large enough to derive specificity estimates. The specificity of IRT (cut off ≥60 μg/l) was 98.99%. Specificity of PAP (cut off ≥1.6 μg/l if IRT ≥100 μg/l and ≥3.0 μg/l if IRT ≥60 μg/l) was 89.99%, Figure 5.1.[4]
The sensitivity could be determined relative to another strategy. Moreover, the number of CF patients detected was too small for reliable estimates of sensitivity (Figure 5.1). Therefore, we also used data from other studies. Sensitivity of IRT in the French nationwide NBSCF program among 2,7 million infants was 95.6% at 60 μg/l

(95% CI 93.3-97.1%,[16] and additional personal communication). We found a sensitivity for IRT of 100% (95% CI 80-100%).[4]

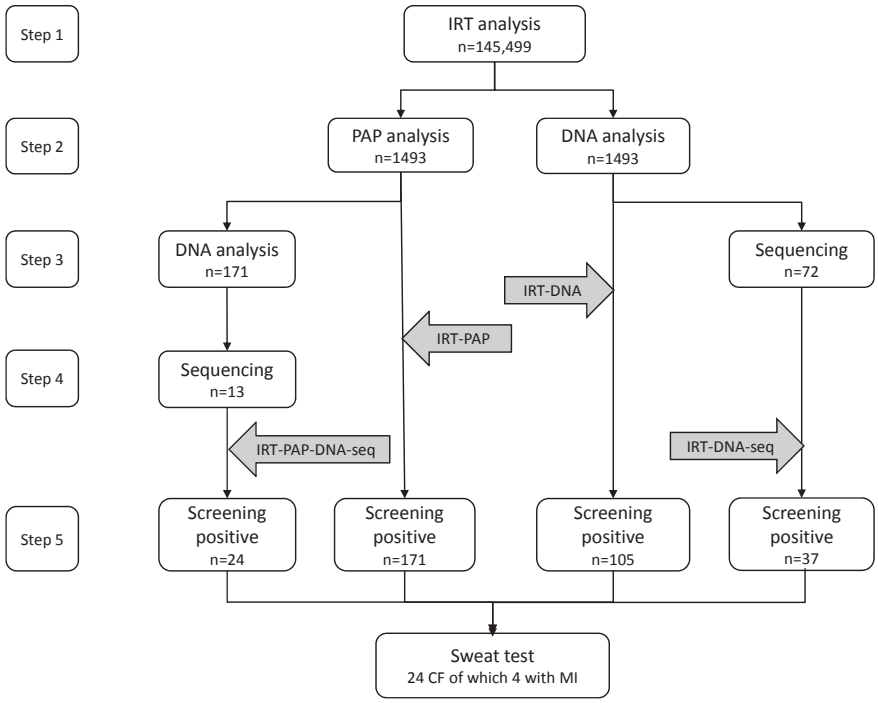

Figure 5.1 Flow diagrams for 4 screening strategies, numbers derived from CHOPIN study between parentheses.
Step 1. All 4 screening strategies start with IRT-analysis. When the IRT-concentration is below cut-off level, the test is negative; when the IRT concentration is elevated a second step follows.
Step 2. This is either a PAP-analysis or a DNA-analysis. When the PAP-concentration is below cut-off level , or when no CF-causing mutation is found in the DNA-analysis the test is negative. The PAP-test is positive in the IRT-PAP strategy and IRT-PAP-DNA-seq strategy when PAP-concentration is above cut-off level; and the DNA-analysis is positive in the IRT-DNA strategy when one or two CFTR mutations are found, and in the IRT-DNA-seq strategy when two CFTR mutations are found, but when only one mutation is found a third step follows.
Step 3. In the IRT-DNA-seq-strategy the test is negative when only one CFTR mutation is found after sequencing, and positive when a second CFTR mutation is found. In the IRT-PAP-DNA-seq strategy this step, DNA-analysis, follows when both IRT and PAP concentrations are elevated. The test is negative when no CFTR mutations are found, positive when two CFTR mutations are identified, when only one CFTR mutation is found sequencing follows in step 4.
Step 4. Sequencing. The test is negative when after sequencing only one mutation is found, and positive only when a second CFTR mutation is found.
Step 5. All children that are screen-positive (screen+) are referred for a sweat test. CF diagnosis is confirmed when Cl≥60, excluded when Cl<30, equivocal when between 30 and 60 mmol/L.
CHOPIN=Cystic fibrosis Heel prick amOng a newborn Population In the Netherlands, IRT=immunoreactive trypsinogen, PAP=pancreatitis-associated protein, DNA=desoxyribo-nucleic acid, CF=cystic fibrosis, MI=meconium ileus

Sensitivity of PAP was 100% in several small studies.[2,17] This might be an overestimate. Therefore, we used 95% as a base-case value. Sensitivity of the mutation panel to find at least one mutation in CF-patients was 100% in the 20 CF patients without MI and 98.6 % in retrospectively tested dried blood spots of CF patients (n=74, 95% CI 91.6-99.9%).[4] Sensitivity of sequencing was assumed to be 100%, as all seven samples of CF patients with one mutation at the DNA-test were retrospectively found to have two mutations at sequencing. In earlier studies, 100% of 10 patients and 10 carriers with CF and 100% of 72 patients with CF were accurately identified by the application of extended gene analysis and the Ambry test respectively.[18,19]

Results of DNA-test and sequencing

4.6% of IRT-positive samples of non-CF-patients had one CFTR mutation at the DNA-test, and needed sequencing in the IRT-DNA-seq strategy(Figure 5.1). For IRT-PAP-DNA-seq , 5.4 % IRT- and PAP-positive samples of non-CF-patients were sequenced.[4]
Among the 20 CF patients, 17 had two CF-mutations identified by the DNA-test, but in three patients (15%, 95% CI 4-39%) only one CFTR mutation was identified initially. Sequencing identified the second mutation. In retrospectively tested dried blood spots of children with CF, sequencing was necessary in 10% (95% CI 4-19%).[4] We assumed that in 11% of CF-patients further gene analysis will be necessary to detect the second mutation.

Health effects

We assumed a relative reduction of 25% in mortality rates among children with CF diagnosed by NBS compared to children with a clinical diagnosis of CF.[20,21]
We assumed a gain of 40 life years per CF death prevented by NBS. No gain in life expectancy was assumed for CF patients with MI, as these infants are diagnosed shortly after birth, and therefore do not benefit from NBS. The number of quality adjusted life years gained is the preferred effect measured in cost-effectiveness analyses. Only two studies on health–related quality of life and NBS were found.[11,22] We, therefore, used the number of life years gained as effect measure in this study.

Costs

Based on costs of reagents, equipment and technical and administrative personnel, the costs of IRT were calculated to be €2.28 per test (L.H. Elvers, expert opinion), assuming that a blood spot has been collected for other newborn screening tests. PAP was performed on 96 wells anti-PAP coated plates once a week (80 samples per plate). The capacity of PAP-testing in the five screening laboratories is therefore more than 20,000/year, while for a population of 185,000 newborns only about 1,900 tests are

needed. Yearly costs are therefore almost independent of the number of PAP-tests, and are €294,413.

Costs of multiple mutation DNA and sequencing were respectively €166 and €417 (J.J.P. Gille, expert opinion). These costs include the costs of DNA extraction, DNA analysis, laboratory space, equipment, reagents, supplies, licenses, and technical and administrative personnel. As fixed administration and equipment costs need to be distributed among a smaller number of DNA-tests in IRT-PAP-DNA-seq, costs per DNA-test were higher for this strategy (€231).

In the IRT-(PAP)-DNA-seq strategies infants with one CFTR mutation were healthy carriers and screen-negative (Figure 5.1). 87% of Dutch parents wanted to be informed about the carrier result of their child if screening would reveal this.[23] Almost 50% indicated that they want to be tested whether they were a CF carrier themselves if their child would be a carrier. The percentage of parents accepting genetic counselling for CF is unknown at this moment. After genetic counselling for CF the uptake of genetic testing in other countries varies from 22-85%.[24-26] Baseline assumption in the model is that 50% of parents of carriers would accept genetic counselling, and 80% would subsequently be tested. Costs of genetic counselling are €515 per couple and if they decide to be tested, the additional costs are €740 per person, both amounts include travel (7 km) and time (2 hours) costs.[27,28]

Costs of integrating NBSCF in the existing NBS programme were calculated as earlier.[29] These costs amount to €153,716 per year. Costs for diagnosis of screen-positive newborns with MI were not attributed to the screening program.

Costs of a first sweat test amount to €274. These costs consist of referral to a CF-centre (60 minutes), one outpatient visit to the paediatrician and GP, time of a CF-nurse (90 minutes), laboratory costs, and travel and time (120 minutes) costs of both parents.[27,28] 1.17 sweat tests per screen-positive child were needed on average.[4] Costs of a second or third sweat test were €206 as referral and consultation of the GP were not needed.

Without NBSCF generally several diagnostic tests are performed before the diagnosis CF is made. We assumed average diagnostic costs of €9,986 (indexed from 1) per patient. In a situation without screening, for each clinical CF patient diagnosed about 100 sweat tests are performed in children without CF.[1,30] After introduction of NBSCF, the number of sweat tests may reduce. A decreasing number of sweat tests was observed after introduction of CF screening in the UK in 2007.[31] A steady state seems not to have been reached. The number of sweat tests after introduction of NBSCF was assumed to eventually be 50% of the number of sweat tests without screening. In a sensitivity analysis also values of 10% and 100% (no change) were used.

Yearly costs of care were €34,839 for children up to 18 years of age, and €45,564 for adults, based on present guidelines,[32] frequency of complications from the Dutch CF

registry 2007, and 20% overhead costs (V. Gulmans, expert opinion). They exclude costs of home care, which were added (€5,042 (indexed from the study of Wildhagen[33]) and costs of hospitalisation in case of complications. Estimates based on declarations at health insurance companies are about €10,000 smaller. Lifetime costs of care of a CF patient are estimated at €895,291 (based on declarations) to €1,154,122 (based on guidelines and data from the Dutch CF registry, both costs with 3% discounting). We assumed similar treatment lifetime costs of care for patients detected by screening, but also savings of 5%.[34] Costs in life years gained due to NBSCF were not included in the analysis.[1,6]

Base case analysis and sensitivity analysis

In Table 5.1 the values of the model parameters used in the base case model are presented. Base case values are based on data from the CHOPIN study, or best estimates from literature as described above.

Sensitivity analyses were performed in which the values of the model parameters were varied. In this way, the degree of influence of each parameter on the cost-effectiveness was studied, and crucial parameters determining the cost-effectiveness of NBSCF were identified. Both univariate and multivariate sensitivity analyses were performed. In univariate sensitivity analyses, one model parameter at a time was varied, while in the multivariate sensitivity analyses all model parameters were varied together. Under the assumption that all of the model parameters mentioned in Table 5.1 are independent from each other, we constructed a set of extreme parameter values that yield the highest and the lowest cost-effectiveness ratios. Furthermore, we performed a probabilistic multivariate sensitivity analysis. In the probabilistic multivariate sensitivity analysis we assumed distributions for the parameters as presented in Table 5.1. Uniform and triangular distributions varied between the lower and upper value presented, while for the binomial and Poisson distributions the upper and lower value of the 95%-confidence interval are presented in Table 5.1. Subsequently, 50,000 random draws from probability distributions defined for the model parameters were taken for each model parameter, and the resulting cost-effectiveness ratio for these parameter values is calculated using @RISK, Version 5.7 (Palisade Corporation). In this way acceptability curves were constructed showing the proportion of random draws for which a screening strategy is optimal as a function of the willingness to pay (λ). In this case, an intervention is optimal for a particular random draw when it is associated with the maximum net benefit (net benefit : [λ x life-year gained] - cost). Also, information was derived about the influence of the uncertainty around the individual parameters on the uncertainty in the cost-effectiveness ratio.

Results

Table 5.2 presents the expected results. In a situation without screening, 32 children with CF without MI were expected to be born annually. IRT-PAP as well as IRT-PAP-DNA-seq would detect 29 CF-infants, and IRT-DNA-seq and IRT-DNA 30 infants with CF. After IRT, 1862 samples would be positive. Table 5.1 shows the results of further testing for the four strategies.

The additional costs of implementing NBSCF ranged from €213,000 to €272,000 per year (Table 5.2). IRT-PAP was the cheapest, even though costs of diagnostics of screen-positive children were highest. The other strategies were more expensive due to higher costs of screening, and the costs of genetic counselling when detecting a carrier.

Compared to a situation without screening, strategies had cost-effectiveness ratios (CER) varying from €23,600 to €29,200 per life-year gained. However, IRT-PAP-DNA-seq was dominated, as more effects could be obtained for lower costs with the IRT-PAP. Also IRT-DNA-seq was dominated, as it resulted in the same number of life years gained as IRT-DNA, however for higher costs. IRT-PAP had the most favourable CER of €23,600 per life-year gained compared to 'no screening'. Additional life years could be saved by IRT-DNA. Relating the additional life years to the additional costs of IRT-DNA compared to IRT-PAP, led to incremental costs of €147,600 for IRT-DNA compared to IRT-PAP.

Table 5.3 shows the results of the univariate sensitivity analyses. Changes in the lifelong costs of clinically diagnosed patients did not affect the CER of screening, when the lifelong costs of patients identified by screening changed accordingly. Assuming that the lifelong costs of treatment of patients detected by screening are 5% lower than the lifelong cost of clinically diagnosed patients NBSCF would result in both financial savings and years of life gained. The reduction in the yearly number of sweat tests after introduction of CF-screening also largely affects the costs. With a 10% reduction in the number of sweat tests after implementation of NBSCF, CF-screening would result in both financial savings and years of life gained, while CERs are around €75,000 per life-year gained if the number of sweat tests does not reduce.

Constructing two sets of extreme parameter values resulting in the least favourable and most favourable cost-effectiveness, showed that the CER of adding screening for CF to the Dutch newborn screening programme ranged between €262,000 per life-year gained for IRT-PAP and €305,900 for IRT-DNA-seq in the least favourable situation, to both financial savings (range €2.3 million to 2.4 million) and life years gained for all strategies (36.0 life years) in the most favourable situation.

Table 5.1 Model parameters: base case values and lower and upper values in sensitivity analysis, and distribution for the probabilistic multivariate sensitivity analysis.

a

Model parameter	Base-Case Value	Lower value Sensitivity Analysis	Upper value Sensitivity Analysis	Distribution
Annual births (n)	185,000			
Participation NBSCF (%)	99.7	99.5	99.8	Triangular
Incidence of classic CF (%)	0.021 (1 per 4,750)	0.018 (1 per 5,500)		
Newborns with meconium ileus (%)	17	15	19	Triangular
Sensitivity IRT-test (cut-off 60 µg/l) (%)	95.6	90	100	Triangular
Sensitivity PAP-test (%)	95	90	100	Triangular
Sensitivity DNA-test (%)	99	95	100	Binomial
Sensitivity sequencing (%)	100	97		Uniform
Specificity IRT-test (cut-off 60 µg/l) (%)	98.99	98.94 (95%CI)	99.04	Poisson
Specificity PAP-test (%)	89.99	88.32 (95%CI)	91.46	Binomial
Specificity DNA-test (%)	100			
Specificity sequencing (%)	100			
Infants with positive IRT test having 1 mutation at DNA-test (%)	4.6			
Infants with positive IRT-PAP having 1 mutation at DNA-test (%)	5.4			
CF patients with 1 mutation detected by DNA-test (%)	11	5	17	Binomial
CF mortality in childhood (%)	6	3	10	Triangular
Reduction in childhood CF mortality due to screening (%)	25		50	Uniform
Life years gained per prevented death due to screening (3% discounting) (years, standard deviation)	40 (20.5)	35 (19.4)	45 (21.8)	Triangular
Parents opting for genetic counselling (%)	50	40	90	Triangular
Parents testing carrier status after genetic counselling (%)	80	50	90	Triangular

b

Model parameter	Base-Case Value	Lower value Sensitivity Analysis	Upper value Sensitivity Analysis	Distribution
Costs (€)				
Adding CF screening to neonatal screening program	153,716	100,000	200,000	Triangular
IRT test	2.28			
PAP test	294,413 for a year cohort (155 per test)	200,000		Uniform
DNA test	166 (IRT-DNA) 231 (IRT-PAP-DNA)			
Sequencing	417			
Sweat test	274 (1st test) 206 (repeated test)			
Intestinal current measurements	1,419			
Genetic counseling	515			
Testing for carrier status, per couple	1,479			
Clinical diagnosis CF	9,986	8,000	12,000	Triangular
Lifetime costs of treatment for clinically diagnosed patient	895,291	750,000	1,200,000	Triangular
Sweat tests per screen positive child (n)	1,17			
Savings in life time costs of treatment due to screening (%)	0		5	Uniform
Sweat tests for diagnosis of non CF patients per clinically diagnosed CF patient (n)	100 without screening With screening 50% of no. without screening	With screening 10% of no. without screening	With screening 100% of no. without screening	Triangular

CF=cystic fibrosis, IRT=immunoreactive trypsinogen, PAP=pancreatitis-associated protein, DNA=desoxyribonucleic acid

Table 5.2 Overview of the predicted costs and effects of newborn screening for cystic fibrosis in the Netherlands for a cohort of 185,000 children (3% discounting)

	No screening	IRT-PAP	IRT-DNA-seq	IRT-PAP-DNA-seq	IRT-DNA
Screen-positives (n)		222	53	37	138
CF, with MI[*] (n)		6	6	6	6
CF, no MI (n)		29	30	29	30
False-positives excl. equivocal diagnosis (n)		182	0	0	88[†]
Equivocal diagnosis (n)		4[‡]	17[§]	3[§]	14[§]
Carriers (n)		0	85	10	88[†]
Life-years gained (years)		9.0	9.3	8.9	9.3
Costs (€x1,000)					
Organisation		154	154	154	154
Screening		714	773	772	735
Diagnosis		78	18	10	45
Genetic counseling for carriers		0	72	9	74
Clinical diagnosis of missed CF cases	322	30	19	34	19
Diagnosis of non-CF patients (sweat test)	883	441	441	441	441
Treatment for patients identified by screening		26,144	27,148	25,791	27,148
Treatment for clinically diagnosed patients	28,872	2,728	1,724	3,081	1,724
Total (€x1,000)	30,077	30,289	30,349	30,292	30,340
Additional costs compared to no screening (€x1,000)		213	272	216	263
Costs per life year gained (€)		23,600	29,200	24,300	28,200

[*] Excluded from the analysis; [†] Only in the IRT-DNA strategy, carriers are false-positives. In this strategy, equivocal diagnosis remains undetected in 3 children with 1 mutation, as sequencing for the second mutation is not performed. [‡] IRT-PAP-positive newborns with equivocal sweat test, who needed further diagnostic tests (DNA-analysis). CF=cystic fibrosis, MI=meconium ileus, IRT=immunoreactive trypsinogen, PAP=pancreatitis-associated protein, DNA=desoxyribonucleic acid, seq=sequencing, n/a=not applicable, dominated screening strategy. [§] newborns with 2 CFTR mutations of which one or both have unclear clinical consequences, and a normal or equivocal sweat test result. [¶] Compared with IRT-PAP

In the multivariate sensitivity analysis, the uncertainty about whether screening for CF will lead to savings in lifelong costs of treatment determines the variance in the CER most. Other parameters that largely affect the cost-effectiveness are the lifelong costs of treatment of clinically diagnosed patients, the reduction in number of sweat tests, and the mortality in early childhood due to CF in a situation without screening, the reduction in childhood mortality as a result of NBSCF, and cost of clinical diagnosis CF.
Figure 5.2 shows the cost-effectiveness acceptability curves for the four newborn screening strategies, including the situation of no newborn screening. At all values for willingness to pay until €100,000 per life-year gained, the IRT-PAP strategy has a higher probability of being cost-effective than all other strategies.

Table 5.3 Univariate sensitivity analysis: Cost-effectiveness ratios for lower and upper values of model parameters included in sensitivity analysis.

Model parameter	IRT-PAP		IRT-DNA-seq		IRT-PAP-DNA-seq		IRT-DNA	
	low	high	low	high	low	high	low	high
Incidence CF (n)	41,300		47,300		42,100		46,200	
Newborns with meconium ileus (%)	20,900	25,900	26,400	31,600	21,600	26,600	25,400	30,600
Participation neonatal screening	23,600	23,600	29,100	29,200	24,300	24,300	28,200	28,200
Sensitivity IRT test	27,000	21,200	32,900	26,500	27,700	21,900	31,900	25,600
Sensitivity PAP test	26,700	20,900	BL	BL	27,400	21,500	BL	BL
Sensitivity DNA test	BL	BL	31,500	28,300	26,400	23,500	30,500	27,400
Sensitivity sequencing	BL		29,400		24,500		BL	
Specificity IRT test	24,000	23,300	31,100	27,300	24,500	24,100	30,100	26,400
Specificity PAP test	24,800	22,600	BL	BL	25,000	23,700	BL	BL
Infants with positive IRT test with 1 mutation at DNA-test (%)	BL	BL	26,700	32,300	BL	BL	26,000	31,100
Infants with positive IRT-PAP with 1 mutation at DNA-test (%)	BL	BL	BL	BL	23,500	25,700	BL	BL
CF patients with 1 mutation detected by DNA-test (%)	BL	BL	29,100	29,300	24,200	24,400	28,100	28,300
CF mortality in childhood	47,200	14,200	58,300	17,500	48,600	14,600	56,400	16,900
Reduction in childhood CF mortality due to screening		11,800		14,600		12,100		14,100
Lifeyears gained per prevented death due to screening (years)	25,000	22,300	30,900	27,500	25,700	22,900	29,900	26,600
Parents opting for genetic counselling (%)	BL	BL	27,600	35,300	24,100	25,100	26,600	34,600
Parents testing carrier status after genetic counselling (%)	BL	BL	27,100	29,900	24,000	24,400	26,100	28,900
Costs of adding CF screening to neonatal screening programme	17,700	28,800	23,400	34,100	18,200	29,500	22,500	33,200
Costs of PAP test	13,100		BL		13,700		BL	
Costs of clinical diagnosis CF	30,100	17,100	35,600	22,600	30,700	17,800	34,700	21,700
Lifetime costs of treatment for clinically diagnosed patient	BL	BL	BL	BL	BL	BL	BL	BL
Savings in life time costs of treatment due to screening (€x1,000)		-1,095		-1,085		-1,074		-1,094
Sweat tests for diagnosis of non CF patients per clinically diagnosed CF patient (n)	Savings €-141,000	72,700	Savings €-81,000	76,400	Savings €-137,000	74,000	Savings €-92,000	75,500
Baseline	23,600		29,200		24,300		28,200	

CER=costs (€) per life year gained. When screening resulted in cost savings compared to a situation without screening, CER has no meaning. Instead, cost savings (in €) due to screening are presented. In these cases, life years gained were equal to the values presented in Table 5.2. CF=cystic fibrosis, IRT=immunoreactive trypsinogen, PAP=pancreatitis-associated protein, DNA=desoxyribonucleic acid, seq=sequencing, BL=results are equal to the results under baseline assumptions (last row)

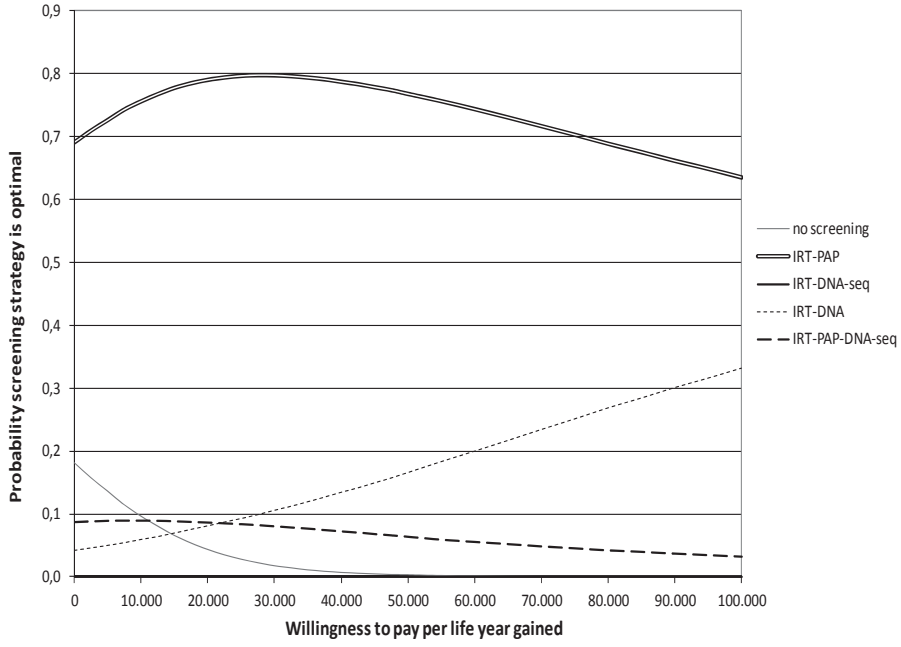

Figure 5.2 Cost-effectiveness acceptability curves of the different neonatal screening strategies for cystic fibrosis, including no neonatal screening.
IRT=immunoreactive trypsinogen, PAP=pancreatitis-associated protein, DNA=desoxyribonucleic acid, seq=sequencing

Discussion

Our study using primary data from the CHOPIN study showed that IRT-PAP had the most favourable CER of €23,600 per life-year gained. Additional life years can be gained when using screening strategies incorporating DNA. The incremental costs of the additional life-years mount up from €147,600 per life-year gained for the IRT-DNA strategy compared to the IRT-PAP strategy. IRT-PAP-DNA-seq and IRT-DNA-seq were dominated by the other screening strategies resulting in similar or less life-years saved at comparable or higher costs.

Calculations in our earlier study were based on data derived from the literature. Changed insights of CF incidence, costs of sweat tests and especially lifetime costs of treatment of CF patients (doubled according to more recent data) resulted in higher total costs in the situation without screening (30 versus 20 million euros). Costs per life-year gained due to CF screening were smaller in the present study, due to reduced costs of IRT tests and reduced number of sweat tests needed for non-CF patients after introduction of screening for CF.

Costs for IRT-DNA were assessed in a decision tree approach at $678,000 ($2010 US) for a cohort of 100,000 children, i.e. around €500,000.[35] Rescaling our results on costs included in this study (costs on screening, diagnosis, genetic counselling and clinical diagnosis of missed CF patients) to a cohort of 100,000 results in a comparable figure of €470,000. A more favourable CER of IRT-DNA was observed earlier,[34] but we included more cost elements (organisation of CF screening, genetic counselling) at higher cost estimates.

Some parameters still were assessed on the basis of literature and expert opinions. Sensitivity analyses showed that uncertainty on reduction of lifelong costs of treatment of CF patients detected by screening affected the cost estimates to a large extent. The uncertainty about the reduction in the yearly number of sweat tests after introduction of CF-screening also affects the cost estimates.

We worked with an overcapacity of PAP, with 80 well plates. After introduction of 10 well plates in the future, the IRT-PAP and IRT-PAP-DNA-seq strategies will be more cost-effective.

Some elements were not incorporated in the CERs[1]. Firstly, we used the number of life-years gained as effect measure, while the preferred outcome measure would have been the number of quality adjusted life-years gained. However no good parameters of the effect of NBS on quality of life for CF are available.[11,22] It is undisputed that NBS leads to improved growth and nutritional status, and solid evidence is arising for a longstanding positive effect on lung function and survival in young adulthood.[36] Our baseline assumption of a 25% reduction in childhood CF mortality by NBSCF may therefore be conservative and cost-effectiveness higher than indicated. Secondly, costs and effects of changes in reproductive decisions were not included in the CER's.[37-40]

Conclusion

This study confirms that NBSCF is economically justified. Its introduction may even result in cost savings when treatment costs of CF patients are smaller after early detection, or when the number of sweat tests used for diagnosis of children with CF-like complaints decreases due to clinicians' awareness that the incidence of undetected CF is very low in screened birth cohorts. The results of our study may help other regions to decide on implementing NBSCF.

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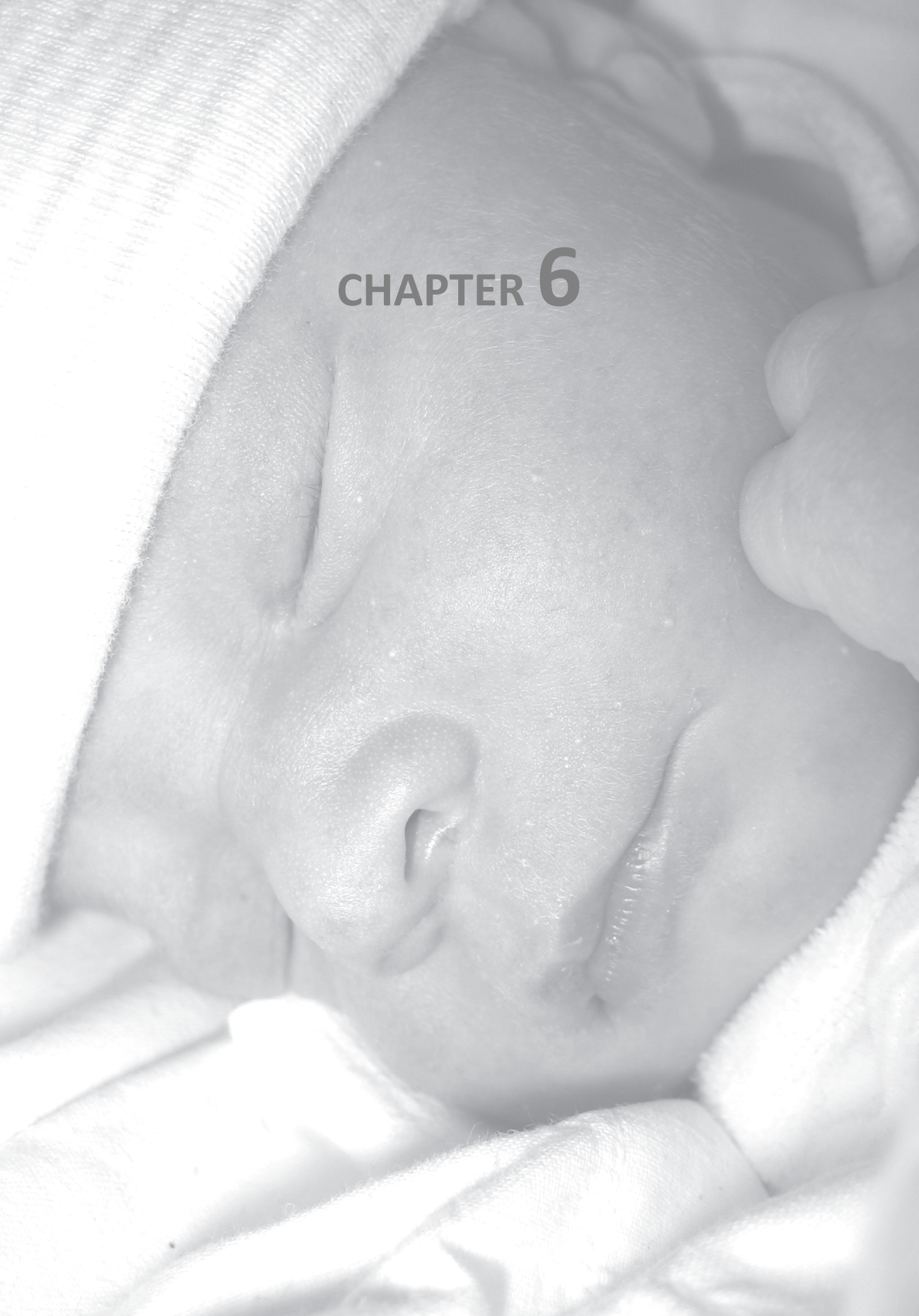

CHAPTER 6

Parental knowledge reduces long term anxiety induced by false-positive test results after newborn screening for cystic fibrosis

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Abstract

Background

False-positive screening results in newborn screening for cystic fibrosis may lead to parental stress, family relationship problems and a changed perception of the child's health.

Aim of the study

to evaluate whether parental anxiety induced by a false positive screening result disappears after six months and to assess whether a special program to inform parents prior and during the screening procedure prevents or diminishes parental anxiety.

Methods

Prospective controlled study assessing the long term effects of false-positive test results of newborn screening for cystic fibrosis (NBSCF) on parental anxiety and stress by means of questionnaires sent to parents of 106 infants with a false positive newborn screening test and 318 randomly selected infants with a true negative screening test. Additionally we interviewed 25 parents of the false-positive group.

Results

Parents showed negative emotional feelings after being informed about the positive screening test result. After confirmation that their child was healthy and not suffering from CF, most parents felt reassured. After six months no difference in anxiety levels between both groups of parents was found. Well-informed parents in the false positive group experienced less stress.

Conclusion

A positive screening test result induces parental anxiety but false positive test results in NBSCF do not seem to cause long-term anxiety. Well-informed parents show lower stress and anxiety levels.

Introduction

Newborn screening for cystic fibrosis (NBSCF) is implemented in many countries worldwide with a variety of screening programs.[1,2] False-positive newborn screening results may lead to parental stress, family relationship problems and a changed perception of the child's health.[3-5]
Long-lasting parental stress after false-positive results in newborn screening has been described for various NBS-programs.[3,5-8] Only two of these studies were controlled. In the first study mothers who had received a false-positive result, showed more anxiety and stress than the control group. Children with a false-positive result were also twice as often admitted to the hospital. However, in this study the age of the infants in both groups differed which may have had a substantial effect on the results.[8] Another controlled study found that false-positive results after newborn hearing screening did not cause long-term parental anxiety in the majority of the parents.[6] During a study comparing two novel strategies for NBSCF we investigated whether or not increased parental anxiety induced by a false-positive screening test persisted after six months.[9]
A secondary aim was to assess if a special education program to inform health care workers and parents prior and during the screening program could prevent or diminish parental anxiety.

Methods

Screening program

This study was part of a larger study investigating the effectiveness of two novel screening strategies in the Netherlands.[9] In only one of these strategies false-positive screening tests were found. This strategy consisted of measurement of immunoreactive trypsinogen (IRT) followed by determination of pancreas-associated protein (PAP).
All newborns with a positive screening test were referred to one of four participating CF-centres for a sweat test to confirm or to exclude the diagnosis (Erasmus Medical Centre, Rotterdam, University Medical Centre Utrecht, Maastricht University Medical Centre, University Medical Centre Nijmegen).
The general practitioner (GP), or paediatrician when the baby was hospitalized, informed the parents about the positive screening test and the sweat-test appointment (24 to 48 hours later). The sweat test was performed not earlier than at a gestational age of 38 weeks and a weight of more than 2000 grams. Parents were informed about the sweat test result the same day or the day after, mostly by telephone by one of the staff members of the CF centre.

Educational material

An information leaflet about NBS for CF was developed and pre-tested by eight pregnant women during an appointment with their midwife in a practice in Zoetermeer, the Netherlands. Parents were asked to read the leaflet and comment on its clearness, and whether they missed information or had additional questions. Their knowledge about CF screening was also tested after reading the leaflet.
The leaflet informed parents about CF, screening, genetics, the opting-out procedure, privacy and links to websites for more information. The leaflet was printed in Dutch (also available in nine languages on the internet) and was distributed to parents along with the leaflet of the routine newborn screening program three times: 1) in the third trimester of the pregnancy by their midwife/gynaecologist, 2) at the registration of their child's birth at the city hall, and 3) when the heel prick was performed.
Before the screening program started we educated the healthcare workers involved in NBSCF (midwifes/gynaecologists/general practitioners/performers of the heel prick/paediatricians). Group sessions were held in all regions, with a presentation about NBSCF and group discussions. All health care workers received a letter with information about the study, the screening program and cystic fibrosis (CF). After a positive screening test, the GP received written information by e-mail or fax about the study, the probability of a false-positive test result, CF and the sweat test. The GP also received written information to give to the parents.
The sweat tests were done in the CF-centers, where parents had an appointment with a special trained CF-nurse and a paediatric pulmonologist.

Study population

All parents of infants with a false-positive screening test result for CF (normal sweat test result; chloride concentration <30 mmol/l) born in 2008 were invited by letter to complete a questionnaire. Infants with a lethal congenital condition or infants with a positive screening test for another disease were excluded, whereas parents who did not want further participation in the study were not invited.
The three card numbers, following the heel prick card of the infant with a false positive result were used to compose a control group (C) of parents of screen-negative infants. When a false-positive result was known the parents were contacted by our research-nurse (SR) by telephone to ask if they were willing to participate in an interview.

Study design

This was a prospective controlled observational study. All parents of babies with a false-positive screening result (FP group) and control (C) group received a questionnaire at home six months after the positive test result. Results were recorded

anonymously. Additional semi-structured interviews were held with 25 parents of the FP group.

Questionnaire

The questionnaire contained items based on a study about the impact of a false-positive result after Medium Chain Acetyl-CoA Dehydrogenase Deficiency (MCADD) screening[10], the existing Hospital Anxiety and Depression Scale (HADS)[11] and items of the TNO-AZL Preschool children Quality of Life questionnaire (TAPQOL)(see appendix).[12,13]

First, parents were asked to complete eleven questions about NBS for CF, based on the information leaflet. Scores ranged from 0 (all wrong) to 11 (all answers correct).

These knowledge items were followed by four questions about how parents assessed the risk of their child being affected with CF, their perceived reliability of the screening test, whether they regretted their participation in NBS for CF and if they would participate again. Scores ranged from 1 (negative) to 5 (positive). Parents were asked whether they were informed about NBS for CF (yes/no), if they were satisfied with the information by different information sources (scale 1-10), and if they had missed any information.

To prevent the questionnaire being too long and to improve the response we decided to use parts of existing standardized questionnaires. We selected 10 questions of the TAPQOL[12,13] concerning the child's health, emotions and behavioral problems in the last three months. The selected health items were based on possible CF-related physical complaints (stomach pain, cramps, airway problems, dyspnoea, bad appetite), behavioral problems (sleeping disturbances, crying) and emotions (happiness). The TAPQOL asks parents how frequent a complaint was noticed (never/sometimes/often). In addition, parents were asked how often their child had been ill compared to other children (more often/ equally/ less frequently), their concern about the child's health (scale 1-5; 1= very concerned 5= not concerned), and how often they had visited their GP.

Parent were also asked to complete four items of the HADS questionnaire, three anxiety items and one item on depression (Scale 1-4; 0= negative to 4= positive).[11]

In addition, parents in the FP group were asked about their experience in the CF-centre during the follow-up tests.

Interviews

Twenty-five additional semi-structured in-depth interviews were done by a research nurse (SR) at the parents' home to investigate the experiences of parents and the impact of the false-positive result in more detail. Parents were selected from the

known false-positive infants, about five parents per 20 false-positive results. The interviews were performed about six months after the false-positive test result. Interviewed parents had also completed the questionnaire.
The semi-structured questions contained six areas; 1) knowledge and education about CF screening, 2) performance of the heel prick, 3) information about the positive screening result, 4) Experiences with the follow-up care, 5) Feelings after the diagnosis was ruled out and 6) Opinions about NBS for CF in general. All interviews were recorded on tape and transcribed. Two researchers (AV and SR) analyzed the results by independently assigning quotes to themes and comparing these themes.

Statistical analysis of the questionnaires

The two groups were compared using the Student t-test for quantitative variables, the Chi-square test for dichotomous variables, and the Mann-Whitney-U or Kruskall-Wallis test for variables on an ordinal scale. In paired series, quantitative variables were compared by using the paired t-test (HADS score after NBS result and six months later). Cronbach's alpha was used to determine whether questions belonging to the same scale could be summed up in a single scale score (Cronbach's alpha of 0.7 or higher). For all tests, a p-value < 0.05 was considered to be significant.
The correlation between being well-informed (high total test score (8-11 out of 11 points)) and the level of stress and anxiety (HADS-sum scale) and the relationship between knowledge and negative feelings after being informed was explored using Spearman correlation.

Results

Study population

There were 109 infants with a false positive screening test for CF in the IRT-PAP program. Parents of three infants with a false-positive result were excluded; two infants died soon after birth and parents of one child declined further participation. Therefore, we sent questionnaires to 106 cases and 318 controls. A total of 62 (59%) questionnaires of the false-positive (FP) group and 146 (46%) of the control (C) group were returned. Both groups had comparable demographic backgrounds, except for the number of mothers that completed the questionnaire in the control group (Table 6.1). Twenty-four mothers and three fathers participated in the 25 semi-structured interviews. The demographic data of this group were not significantly different from the false-positive group as a whole.

Table 6.1 Demographic data of the population.

	False-positive n(%)	Controls n(%)	p value
Total	62 (58.8)	146 (45.9)	0.002†
mother	48 (77.4)	136 (93.8)	
father	10 (16.1)	8 (5.5)	
both	4 (6.5)	1 (0.7)	
Mean age			
(years) (SD; range)	32 (4.7; 19-43)	32 (4.1; 24-41)	0.806‡
Married status			
married	41	94	0.967†
living together	20	48	
single parent	1	3	
Educational level			
no education	2 (3.2)	3 (2.1)	0.133*
primary school	1 (1.6)	0	
secondary school	33 (53.3)	64 (44.1)	
postgraduate	26 (41.9)	78 (53.8)	
Number of children			
1	33 (53.2)	63 (43.4)	0.155†
2	19 (30.6)	66 (45.6)	
3	5 (8.1)	11 (7.6)	
4	4 (6.5)	5 (3.4)	
>4	1 (1.6)	0	
Land of origin			
the Netherlands	57 (91.9)	137 (94.4)	0.536†
other European	1 (1.6)	4 (2.8)	
other non-European	4 (6.5)	4 (2.8)	
First language			
Dutch	53 (85.5)	128 (88.3)	0.297†
Dutch and other language	8 (12.9)	17 (11.7)	
other	1 (1.6)	0	
Do you understand Dutch?			
yes	60 (98.4)	143 (98.6)	0.887*
no	0	2 (1.4)	

*Mann-Whitney-U; †Chi-square test; ‡Student-t-test

Knowledge and education

Parents in the FP group had significantly higher knowledge test scores compared to the C group, with a mean score of 8.6 (SD 1.86) versus 7.5 (SD 2.27) (p=0.014;Table 6.2). Although a trend was found that parents in the FP group searching for extra information had higher scores in the knowledge test compared to parents that did not

look for extra information, this difference was not significant (p=0.051). About a third of the parents was informed about CF screening by their midwife/gynaecologist (Table 6.3) and a third received the leaflet. More parents in the FP group looked for additional information on the internet, compared to the control group (Table 6.3). The additional interviews showed, that although a third (9/25 interviewed parents) of the GP's discouraged parents to search on the internet, almost all (21/25) looked on the internet for additional information.

Table 6.2 Correctly answered knowledge items in the false-positive group and control group.

		FP (n=62) n(%)	C (n=146) n(%)	p value*
1	Early identification and treatment of CF will help to decrease health problems belonging to CF	53 (86)	130 (89)	0.471
2	The risk for a child to have CF is very small (<1%)	28 (45)	64 (43)	0.860
3	Participation to the CF study is obligated for every newborn baby	49 (79)	107 (73)	0.381
4	More blood is needed to perform CF screening	41 (66)	77 (52)	0.075
5	All newborns with a positive test result are affected with CF	60 (97)	89 (60)	<0.001
6	Parents always receive the test result also in the case that the test is negative	48 (77)	127 (87)	0.084
7	Data from this study may only be used for this study on NBSCF	43 (69)	115 (79)	0.381
8	A healthy person can be a carrier of CF	56 (90)	91 (62)	<0.001
9	If the screening result is positive you will be informed by your general practitioner	59 (95)	104 (71)	<0.001
10	During this study, you will also be informed about the carrier status of your child	35 (56)	46 (32)	0.001
11	My child is affected with CF	59 (95)	135 (93)	0.478

* Chi-square test. FP=false-positive group, C=control group (screen-negative), NBSCF=newborn screening for cystic fibrosis.

Overall, the FP group was less positive about the given information compared to the C group, although this difference was only significant for the leaflet (p<0.001) and the midwife/gynaecologist (p=0.045). The FP group gave the highest value for the information on the website, but parents in the FP group (39%) missed information about what would happen after a positive screening result. Parents opinions about being informed face-to-face or by telephone varied; 7/25 interviewed parents were informed by telephone, 18 face-to-face of which five were invited to the doctor's practice and in 13 cases the doctor visited the parents at home. Some parents preferred being informed personally, whereas others got the idea the problem must be very serious if the doctor would pay them a visit.

Nineteen of the 25 interviewed parents had heard of CF prior to the screening. Having heard of CF did not mean that parents knew what the disease implicates. Most parents recalled a shortened life-expectancy and recurrent hospital admissions.

Table 6.3 Information sources and the number of parents that received information by the different sources and their evaluation (score 1-10) of the information they received.

	False-positive (n=62)	Control (n=146)	p value
Midwife/gynaecologist (%(n))	29 (18)	40 (59)	0.155*
score (SD; range)	6.3 (1.19; 3-8)	7.1 (1.40; 1-10)	0.045†
Leaflet (%(n))	60 (37)	53 (78)	0.273*
score (SD; range)	6.7 (1.55; 1-9)	7.4 (0.90; 4-10)	0.001†
Website (%(n))	32 (20)	8 (11)	0.001*
score (SD; range)	7.1 (1.06; 5-9)	7.0 (1.14; 5-8)	0.831†
Screener (%(n))	45 (19)	62 (91)	0.599*
score (SD; range)	6.7 (1.59; 2-10)	7.1 (1.37; 1-10)	0.236†
Satisfied with information (%(n))	36 (22)	47 (146)	0.117*
Knew that child would be tested for CF (%(n))	38 (62.2)	not asked	

* Chi-square test.; † Student t-test

Estimated risk of being affected with CF, reliability of screening test and regret

The majority of parents in both groups estimated the risk of their child being affected with CF low, 95%(FP; 59/62) and 92% (C; 134/146) scored one or two on a five-point scale (1=low 5=high). Parents in the FP group considered the CF screening test less reliable compared to the C group (p<0.001). None of the parents in the C group regretted their participation in the newborn CF screening study, versus 11% (7/62) in the FP group (p<0.001). Most parents in the C group would participate again in a next pregnancy (142/146), while 6% (4/62) of the FP group would not participate again and 16% (10/62) was not certain (p<0.001).

Anxiety and depression

Parents were asked about their feelings of anxiety and depression in the last week before filling out the questionnaire (HADS score, Table 6.4). The Cronbach's alpha was 0.771, therefore we made a sum-HADS score (range 4-16; high HADS scores mean low levels of anxiety and depression) . The mean HADS score was 12.95 (SD 2.47, range 6-16) in the FP group and 13.39 (SD 2.16, range 5-16) in the C group, which was not significantly different (p 0.206). Well-informed parents (knowledge test score 8-11) in the FP group showed significant higher sum-HADS scores (Figure 6.1), even when corrected for the parental educational level, which means they experienced less feelings of anxiety and depression (p<0.018).

Table 6.4 Parental stress and concern (HADS score) six months after NBSCF.

	FP %(n) n=62	C %(n) n=146	p value
I feel tense			0.688
mostly	3 (2)	2 (3)	
often	15 (9)	8 (11)	
sometimes	53 (33)	56 (81)	
never	29 (18)	35 (50)	
I can sit down quietly and feel relaxed			0.195
not at all	5 (3)	3 (5)	
not often	16 (10)	23 (33)	
mostly	50 (31)	37 (53)	
always	29 (18)	37 (54)	
I am worried			0.520
very often	7 (4)	3 (4)	
often	10 (6)	8 (11)	
sometimes, but not too often	40 (25)	44 (64)	
sometimes	44 (27)	46 (66)	
I feel cheerful			0.117
not at all	0	1 (2)	
not often	8 (5)	3 (4)	
sometimes	21 (13)	15 (22)	
mostly	71 (44)	81 (117)	
Mean score (4-16)	12.95	13.39	0.289

Numbers do not always add up to the numbers at the top of the columns due to incomplete filled in questionnaires. P values calculated with Mann-Whitney-U test. FP=false-positive group, C=control group (screen-negative), NBSCF=newborn screening for cystic fibrosis, HADS=Hospital Anxiety and Depression Score.

Emotions after a positive screening test result

We asked the FP group how they felt after being informed about the positive screening result. The Cronbach's alpha was 0.837, therefore we established a sum-scale ranging from 6-30 points (negative to positive). Parents showed strong negative feelings after being informed about the positive screening result, with a mean score of 9.1 (SD 4.93, range 6-30). Six months later, the diagnosis of CF excluded, the mean score increased to 26.5 (SD 5.16, range 8-30). Figure 6.2a and b show the difference between emotional feelings immediately after the positive test result and six months thereafter ($p<0.001$). There was no significant correlation between being well-informed and negative emotional feelings ($p=0.589$).

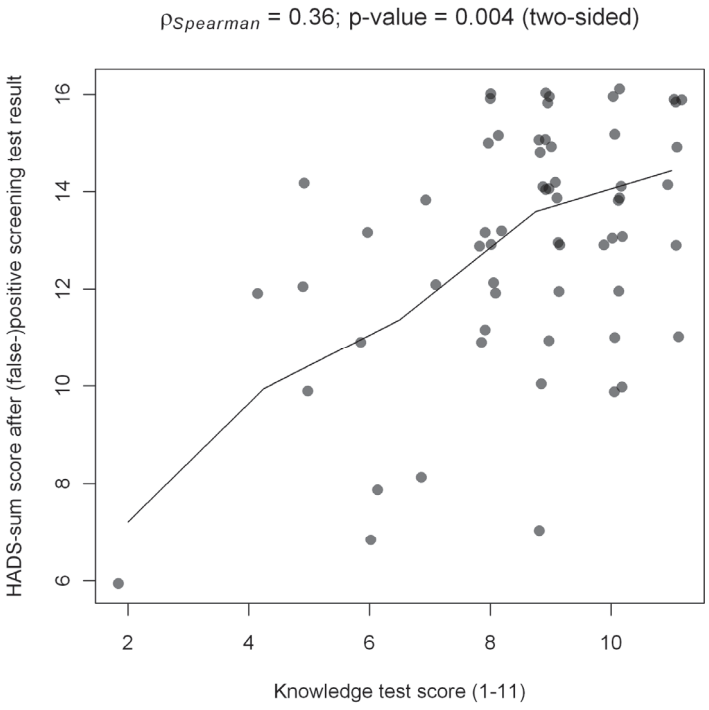

Figure 6.1 The relation between parental knowledge about newborn screening for cystic fibrosis measured by the knowledge test score (range 0-11) and the level of parental anxiety and depression measured by the HADS-questionnaire.
HADS=Hospital Anxiety and Depression Scale

Parents' opinion of their child's health (TAPQOL)

Parents in the FP group expressed similar physical complaints compared to the C group (Table 6.5). Parental concern about their baby's health or the number of visits to their GP did not differ significantly between both groups (p=0.223 and p=0.198 respectively). About half of the interviewed parents (48%) observed symptoms that match with CF, mostly during periods of coughing, shortness of breath or other airway problems. During such periods parents often thought about the screening result, and some were in doubt of the diagnosis. Although almost all (22/25) were sure their child was not affected with CF, five stated they had had some doubts about the diagnosis in the past and three of them still felt unsure.

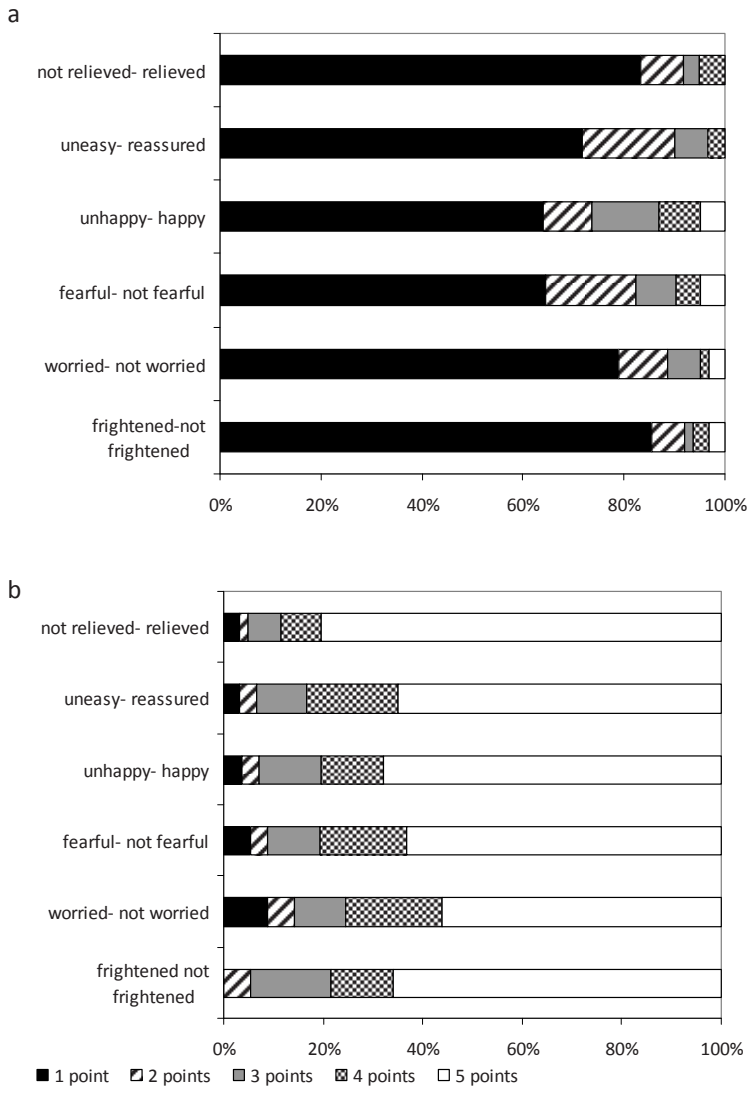

Figure 6.2 Emotional feelings of parents expressed (a) after they were informed about the positive screening test result for CF and (b) six months later, the diagnosis CF ruled out.
After being informed about the positive screening test result, parents were asked to point their emotions and feelings on a five point scale (1= positive, 5= negative). The figure shows the percentage of parents that gave 1 to 5 points respectively.

Table 6.5 Parents opinion about their child's health in the last three months (TAPQOL).

	FP %(n) n=62	C %(n) n=146	p value
Health problems?			0.761
yes	22.5 (14)	20.7 (30)	
no	77.5 (48)	79.3 (115)	
How often did your child suffer from stomach ages?			0.939
never	72.5 (45)	71.1 (101)	
sometimes	22.5 (14)	24.7 (35)	
often	5.0 (3)	4.2 (6)	
How often did your child suffer from abdominal cramps?			0.545
never	36.1 (22)	30.3 (43)	
sometimes	50.8 (31)	59.2 (84)	
often	13.1 (8)	10.5 (15)	
How often did you notice airway problems?			0.838
never	80.3 (49)	80.9 (115)	
sometimes	16.4 (10)	14.1 (20)	
often	3.3 (2)	5.0 (7)	
How often was your child short of breath?			0.836
never	83.6 (51)	80.4 (115)	
sometimes	13.1 (8)	15.4 (22)	
often	3.3 (2)	4.2 (6)	
Sleeping disturbances?			0.521
never	58.1 (36)	50.6 (73)	
sometimes	33.8 (21)	42.4 (61)	
often	8.1 (5)	7.0 (10)	
How often did your child cry at night?			0.058
never	66.2 (41)	48.6 (70)	
sometimes	29.0 (18)	46.5 (67)	
often	4.8 (3)	4.9 (7)	
Were there any feeding difficulties?			0.256
never	82.2 (51)	77.2 (112)	
sometimes	13.0 (8)	20.7 (30)	
often	4.8 (3)	2.1 (3)	
Number of health visits to the general practitioner?			0.198
not visited	40 (25)	56 (79)	
once	34 (21)	30 (44)	
2-3 times	18 (11)	11 (16)	
≥4 times	8 (5)	4 (6)	

Numbers do not always add up to the numbers at the top of the columns due to incomplete filled in questionnaires. P values calculated with Chi-square test. TAPQOL=TNO-AZL Preschool children Quality of Life questionnaire, FP=false-positive group, C=control group (screen-negative)

Follow-up care after a positive screening result

About 88% of the parents was informed by their GP and 12% by a paediatrician. Most parents were satisfied about how they were informed, but 23% was not satisfied. Satisfaction with time of referral was negatively associated with the number of days between being informed and the appointment at the hospital (Spearman correlation

coefficient 0.402; p=0.001). A third of the infants was referred to the hospital the next day, 20% on the second day, 20% on the third day, and about a third of the parents after three days.

Most parents appreciated the way in which they were counseled in the hospital. We saw a trend between negative parental feelings and the number of days between the positive test and the diagnosis, although this was not significant (Spearman p=0.059). The time between performing the sweat test and the result varied; about half of the parents got the result on the same day, 16% the next day, and 34% had to wait two days or longer. In 17% of the infants the sweat test failed and had to be repeated.

Discussion

This study showed that most parents experience strong feelings of concern and anxiety when they receive a positive screening test result for NBSCF. However, six months later, most parents were reassured and sure that their child did not have CF. Parents showed no significant differences in worries about the health of their child compared to a control group of parents of screen-negative infants. Stress-levels were similar in both groups.

Our results confirm the results of a recent non-controlled study showing that the early feelings of anxiety in parents induced by a positive screening result for CF was mostly decreased within three months thereafter, or that parents were reassured after genetic counseling and sweat test results.[14] Results after one and two years did not differ significantly.[5]

Well-informed parents showed less feelings of anxiety and depression. This finding underscores the importance of high quality health education for parents who receive a (false-)positive result. A previous controlled study in NBS for biochemical genetic disorders also showed that parental stress is linked to poor understanding of the test and the disease that was investigated.[3] Some studies show that face-to-face communication leads to better parental understanding, and might prevent misunderstanding of results, especially in low-educated parents and parents with difficulty with the English language.[7]

Despite the specially developed website which was noted in the leaflet and GP-information, part of the GP's discouraged parents to look on the internet, something that parents did anyway. Very few studies report on internet as an information source for parents about NBS.[14-16] Two studies showed that half respectively 82% of the parents use internet after a positive screening result to look for additional information, which is comparable to our study (69%).[16] We think that parents should be guided to reliable and informative websites with information that focuses on the screening itself and some additional information about CF.

The number of health visits during the six months after a false-positive result did not differ from the C group, similar to the findings of another controlled study showing no difference in primary care utilization, emergency room use and hospitalization by the age of six months.[17] Two other studies show a higher hospitalization rate in the FP group.[3,8] However, in these studies the FP group differed from the control group in age respectively in socio-economic status.

Despite their experience with a false-positive test result and follow-up care, many parents were in favor of newborn screening (89%), similar to other studies.[18] Parents were most unsatisfied about not being informed about the possibility and implications of a false-positive test result.

This study differs from most other studies evaluating the effect of a false positive newborn screening test for CF, as no carriers were identified. For some parents, the knowledge of being a carrier leads to misunderstanding, anxiety, and stress.[19] Personal genetic counseling is necessary to remove misunderstanding and concerns about the influence of being a CF carrier on their own health status.[20,21]

Strengths and limitations of the study

The design of this study, prospective, controlled, and of ample size, ensures that the results of our study can be considered as reliable. Also, additional interviews with a small sample of the parents allowed us to receive in depth information about parent's argumentation.

Most responders were born in the Netherlands and spoke Dutch as maternal language. Based on the population data we expected about 8% to be immigrants, of which 56% of non-western origin, which was the case. Therefore multi-ethnicity cannot have influenced the results of our study. However, when interpreting the results we must also consider some limitations. First, the response rate of 59% and 46% was acceptable but not very high. This response rate is common in questionnaire studies, higher response rates are seen in telephone interviews.[3,8,15,21] We decided to use questionnaires so parents could decide whether they wanted to participate in private, and because we could reach a larger group. Second, the educational level of the responders was high and may not be representative for the entire population. Third, the relatively higher number of fathers in the false-positive group might have influenced the results, as mothers are often more anxious about the health of their child compared to fathers.[4] Another limitation to report is that recall bias may have influenced the differences we found about the information parents remembered to have obtained, as the questionnaires were sent about six months after the positive screening test result. However, during the interviews parents appeared to remember this stressful period very well as they described this very detailed. We choose to sent

one questionnaire and not two at different time points because we wanted a high response rate and complete data.

Conclusion

False positive test results for newborn screening for CF not revealing CF carrier status, lead to strong negative feelings immediately after the positive screening test but do not seem to cause long-term parental anxiety. Although, about one fifth of the parents continued to feel worried about their child's health when asked face-to-face. Parents do not assess their child's health differently when compared with a screen-negative control group. Referral within 24-28 hours is important, satisfaction about time of referral was negatively associated with the number of days between being informed and the appointment at the hospital. Well-informed parents showed less anxiety and depression, which shows the importance of providing parents and professionals with adequate educational material. When the parents receive the result of a positive screening test, easy accessible, specially designed websites offer probably the best opportunities for providing the necessary information at the right time.

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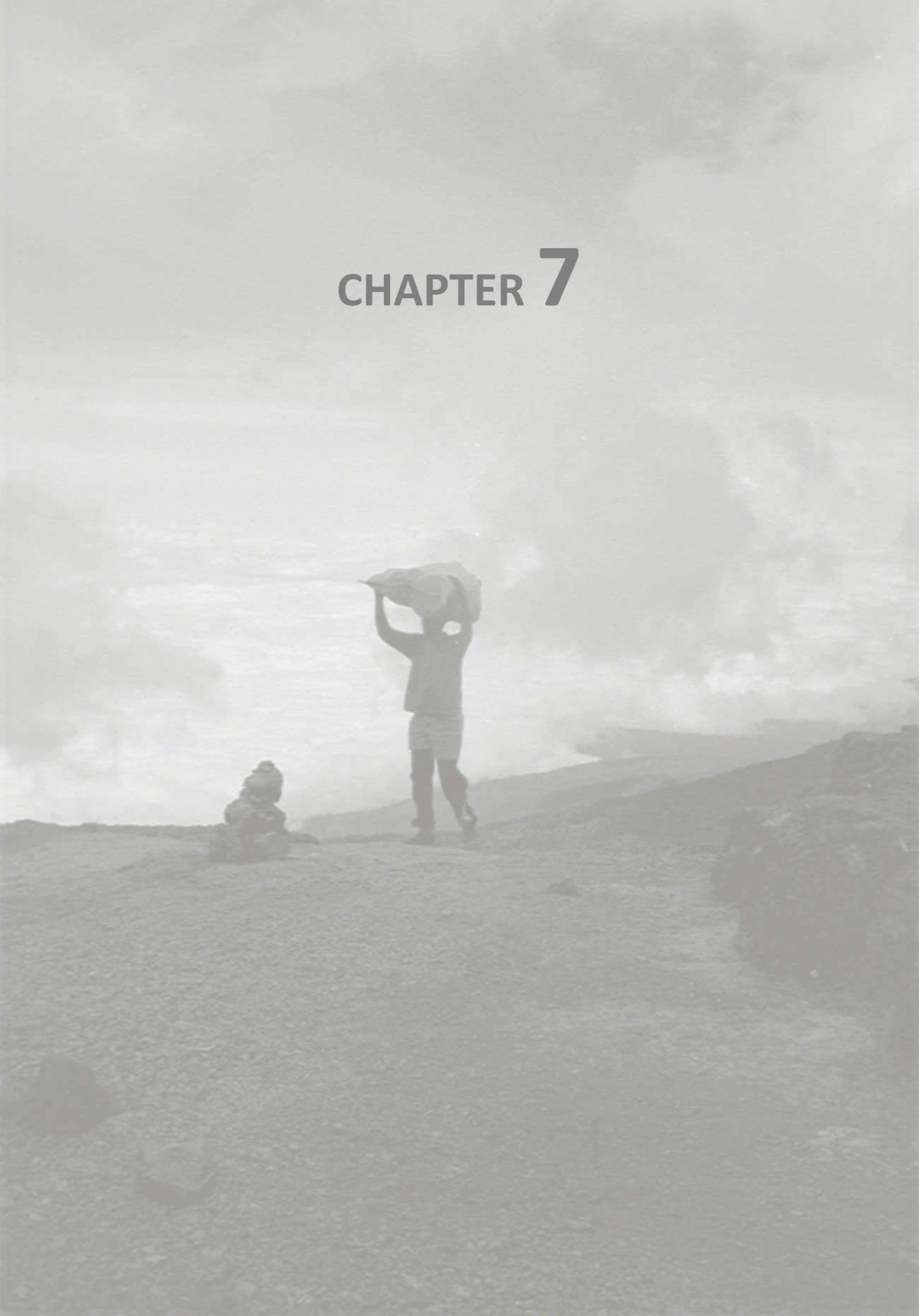
CHAPTER 7



To know or not to know, disclosure of a newborn carrier screening test result for cystic fibrosis

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Abstract

Background

Most newborn screening (NBS) strategies for cystic fibrosis (CF) also identify carriers. However, it is unclear if parents want to be informed about their child's carrier status or not.

Aim of the study

To explore the opinion of parents about disclosure of carrier status of their child when revealed after newborn screening.

Methods

Focus group discussions with pregnant couples to explore their opinions about disclosure of a carrier result for CF of their newborn.

Results

About 87% of parents wanted to be informed when newborn screening would show their newborn being a CF-carrier. Their main reason was the implication of this knowledge for further family planning. Other family members could be informed and children within the family could be tested. Parents stated they have the right to know, but others also expressed that the choice of not being informed should be offered as well.

Conclusion

Most parents want to be informed when NBS for CF reveals that their child is a CF-carrier, but the choice of not being informed should also be offered.

Introduction

Newborn screening programs for cystic fibrosis (CF) aim to identify newborns with CF. Newborn screening for CF (NBSCF) is expanding throughout the world. Screening programs vary but most use a combination of immunoreactive trypsinogen (IRT) followed by either a second IRT at the age of 4-6 weeks or a DNA mutation analysis consisting of one or more cystic fibrosis transmembrane regulator (CFTR)-gene mutations.[1,2] Using a DNA-based program also automatically leads to the identification of unaffected infants that carry one copy of an altered gene for CF; healthy carriers of the disease.

Although the main objective of neonatal screening is early detection and treatment, resulting in considerable health benefits, and not primarily the detection of carriers, informing parents about their newborn's carrier status can be beneficial. The main rationale for informing parents about a carrier test result is the immediate consequence for parent's future reproductive choices. A newborn carrier result implies that at least one of the parents is also a carrier. A blood test can reveal if both parents are carriers and consequently, if they are a couple at risk to give birth to a newborn with CF (25% chance). If it is confirmed that parents are a couple at risk, they have multiple possibilities to prevent the birth of a newborn with CF in case of a next pregnancy (primary prevention). Parents may decide not to have any more children, or they may consider prenatal and pre-implantation diagnosis of CF.[3,4] Secondly, extended family members can be informed and may consider to test themselves before getting pregnant.[4] Finally, other previously born children in the family still asymptomatic may be discovered by sweat testing or DNA analysis.[5]

However, some health professionals consider detection of healthy carriers undesirable because it may cause confusion and anxiety for the parents, and therefore lead to problems in the child-parent relationship and/or early stigmatization.[6] Therefore, carrier identification can be judged a major problem when implementing community-based screening.

Few studies have explored parents' opinion about disclosing a newborn carrier test result.[7] In new strategies for NBSCF carriers are found during the screening program but disclosure of the carrier status is not necessary for the identification of CF patients.[8] Opinions differ whether or not this knowledge should be revealed to the parents.

The aim of this study was to evaluate the opinions and reasoning of future parents about whether or not they wish to be informed when NBSCF reveals that their child is a CF-carrier.

Methods

This study was part of a large study in the Netherlands investigating two novel strategies for NBSCF. In the second strategy in all samples with an IRT above ≥50 µg/l a CFTR-mutation analysis (35 mutations) was performed and when a single mutation was identified an extended DNA analysis was performed by sequencing of the entire CFTR gene. The result of this protocol was positive only when two mutations were detected, results showing only one CFTR mutation were considered screen negative. Therefore parents were not informed when a child was found to be a CF carrier. However, parents were given the possibility to request this information, as was written down in the information leaflet. As part of this study focus groups were organized with future parents to take their opinions into account before implementing newborn screening for CF in the Netherlands.

Study population

Two focus groups were organized after an information meeting for expecting parents in a hospital setting (Atrium Medical Centre) inside the study region where newborn screening for CF was performed, the other two focus groups were organized connected with a pregnant women yoga class in Zoetermeer outside the study region. Participants were asked to join the focus groups, all participated voluntarily but received a small gift certificate afterwards.

Study design

We held dual moderator focus groups, one moderator focusing on the group process and discussion and the second moderator taking notes and documenting nonverbal communication and looking after the environment and logistics.[9-11]
The discussion leader of the focus groups started with an explanation about the disease CF, the newborn screening program, how carriers were identified and the consequences of being a carrier (see appendix). Then participants filled in a short questionnaire individually. Five questions tested if the participant had understood the information. Next, participants were asked for their opinions about seven statements. Those seven statements were discussed in more detail in a group discussion. When all participants indicated having the same opinion during the discussion, the discussion leader would introduce counter-arguments.

Analysis

All focus groups were audio-taped and transcribed. Data were analyzed by two researchers (AR who was also present at the group discussions, and AV), who independently identified key findings under certain themes. Results were discussed

with a senior researcher (JD) and a psychologist-researcher, and also discussion leader of two of the focus groups (SP). Data were de-identified to protect participant confidentiality.

Results

Participants

In total 30 expecting mothers/fathers participated; 23 women and 7 men. Parents participated voluntarily, 8 expecting parents decided not to participate, the reason was not asked for. Most parents were highly educated (44.8% beyond high school and 6.9% completed university). About half of the parents was married (51.7%), the other half was living together (44.8% of which 41.4% registered). One couple had a LAT-relationship (Living-Apart-Together). Twenty-two (75.9%) were expecting their first child, four expected their second child (13.8%), and three parents expected their third, fourth or fifth child respectively (all n=1). All participants were Dutch, except for one woman from Australia. The median age was 29 years, with a range from 21 to 46 years, and the median pregnancy duration was 29 weeks, range 23 to 36 weeks.

Knowledge-items in short questionnaire

The first knowledge statement was that “Cystic fibrosis also is called mucoviscidosis (or “taaislijmziekte”, a much used terminology in Dutch), because very viscous mucus is produced in different parts of the body”. This item was correctly answered with “true” by 26/30 (86.7%) of the parents, 10% thought this was not true and 3.3% had no idea. Next, all parents correctly knew that a healthy person might be a carrier of CF. 90% (27/30) of the parents understood that at least one of them must be a carrier, if their child turned out to be to be a carrier. The same percentage of parents understood that the diagnosis of CF is not sure after a positive heel prick test result, and further tests are necessary. All but one participant remembered that parents were not informed about their child being a carrier during the study on NBS CF in 2008 and 2009. We therefore concluded that participants had understood the information in the introduction well and were sufficiently able to participate in a group discussion about this subject.

Group discussions

We divided the discussion into seven themes and compared the opinions of the participants during the focus groups with their individual responses on the short questionnaire completed prior to the group discussion.

1. "Wanting to be informed about their newborn's carrier test result for CF "

Most participants (26/30; 87%) wanted to be informed about their child being a carrier. Their arguments were that, although a carrier is healthy, the child may use this information when deciding to have children. Parents indicated they would be capable enough to inform their child so that their child could make an informed decision about preconceptional or prenatal screening.

"I think, that we should know, also for the baby's future, to inform him/her (the baby) that (s)he is a carrier." "I think that if that information is known, your child has the right to be informed. On the other hand, (s)he (the child) did not ask for that information and the child is healthy, and therefore the child has no benefit. However, as the child finds a partner who may also be a carrier, and they have a baby with CF, I can imagine that (s)he will say, did you know? I think that when that information is known, the child has the right to hear it."

Another argument was that, if additional screening of the parents would show that both parents are carriers, this would be important information when considering next pregnancies.

"If I would have another child, then I would like to know for a subsequent pregnancy, then we can consider prenatal testing, if that is your choice."

The disclosure of a newborn carrier test result would give parents the opportunity to let themselves tested before a next pregnancy. Another argument was that brothers and sisters of the carrier-child could be tested for CF. *"It may have future implications. And for other children who also have CF but may not have been diagnosed yet."*

About 13% of the parents did not want be informed (4/30), because it had no consequences for the health state of their child and would cause more stress than benefit.

Parents that did not want to have any more children (n=3), were more likely to express that they did not want to know their newborn's carrier result for CF because knowledge of their child's carrier status would not have any direct consequences for them.

Participants in all four groups wanted to have the choice to be informed about their child being a carrier. All agreed to the statement that parents should have the choice to know or not to know and that this should be written down on the heel prick card.

About half of the parents just wanted to know every test result known after the newborn screening of their child.

2. Intention of parents to have themselves tested after a newborn carrier test result for CF.

The majority of participants would test the carrier status of one or both parents (n=14), or would consider testing (n=10). "Indeed, I would consider to test myself and my partner before getting pregnant again." Family planning was the most important element in the answers to this question. Parents planning to have more children would seriously consider to test themselves before getting pregnant again. Some parents would not embark on a new pregnancy when both turned out to be carriers, others would consider prenatal testing. Six participants would not test themselves because they did not want any more children.

"For us, these are the last two, but if we were at the beginning of forming a family, we would want to know if we had a high risk on a child with CF."

3. Informing family members about their newborn's carrier test result for CF

Participants would inform their family, but only direct family (n=17) or family members for whom it may be relevant (n=6). Seven participants would not inform their family, because they thought it was not relevant for their health, or they did not see the benefit of telling them. Motives to inform family members were; possible future pregnancies of their own siblings, and to get support or just to inform family members.

"If this is known, it is a shame if nothing is done with it. But I think that you should not be obliged to inform everyone."

"No, I would not tell everybody, that may not be necessary. Some are very worried and it may not have any consequences for the future. But, maybe I would tell my brother or sister, people for whom it may be useful to know. I would not tell my parents, if he is a carrier it is of no value to let them worry."

"When it would be my sister who received the test result, I would be grateful if I was told about my sisters' child being a carrier."

4. Agreement with the current protocol of the study, wherein a carrier test result is not disclosed to parents.

All parents participating in the focus groups understood that in the present study in only 2% of the newborn blood samples a DNA mutation analysis was performed and that a negative screening test result did not mean their child was no CF-carrier.

Only one participant agreed with the statement that parents should not be informed about the carrier status of their infant during the study.

"I can understand this argument, but if it is known it is a small effort to inform the 2%."

"The results are important, not how many tests are performed."

5. *Agreement with the argument that after implementation of NBSCF carrier results should not be disclosed because a DNA mutation analysis is performed in only a small percentage of the screened population and therefore a negative screening result does never mean the child is no carrier.*

77.8% (21/27) of the parents disagreed with this argument. Participants stated that this information could indeed be difficult to comprehend and could lead to the misconception that a negative screening result means the child is no carrier. Therefore, parental education about the screening program is very important.

"I think it's good, but it may be unclear for parents, they may expect that a negative test means the child is no carrier. That information is difficult."

"I think, if my child has something I always want to know. I just want the best for my child, so if something is going on, I want to know"

6. *Agreement with the argument that the carrier status should not be disclosed because carriers are not affected but healthy.*

About 80% (21/27) of the parents disagreed with the proposition that carriers should not be revealed to the parents because these children are healthy.

"It's true what … (other participant) says. In the future, you can tell your child he's a carrier and other people may benefit from that. I surely want to know. If my child will have children of his own then he may want to take into account that he is a carrier of CF. I think that is important, I want him to have healthy children."

"But suppose your child is a carrier and you have two older children. I would have them tested, to see if they have CF"

7 *Agreement with the statement that parents should have the right to decide they do NOT want to be informed about the carrier status of their child.*

All, but two, participants had the opinion that parents should have the opportunity to choose not to be informed about the carrier state of their child.

"Everybody should have the possibility to decide whether they want to be informed, but for the people that do not want to know, we are not the ones to decide for them."

"I think it's good when the carrier status would be registered at least somewhere in a file or something. That if you ever want to change your mind, or if your child ever wants to know, etc., you will still be able to receive the information. I can say no, but if my child wants to know, (s)he must have the right to receive the information."

Discussion

The aim of this study was to evaluate the opinions and reasoning of future parents about whether or not they wish to be informed when NBSCF reveals that their child is a CF-carrier. Our study showed that 87% of the parents wanted to be informed about the carrier status of their child for the following reasons. First, parents want to have the opportunity to test themselves for their carrier status to determine the risk for CF in subsequent pregnancies and to take well-informed reproductive decisions. Secondly, to inform their child because knowing the risk of being a carrier of CF may influence reproductive choices in his/her future. Third, extended family members could be informed and decide whether or not they want to be tested. A minority of the parents did not want to be informed about the carrier status of their child, because it would not have any future implications for them. All parents agreed that they should be offered a choice whether they want to be informed or not.

Strengths and weaknesses of the study

This explorative study is the first study that reports the opinion of expecting parents about carrier detection as a secondary finding of newborn screening for CF. Focus groups were used to discuss this subject. By using focus groups we had the opportunity to inform the participants about the subject first and were able to confirm they understood the information. Furthermore, the dynamics of a group discussion led to detailed and well-thought motivations. In our study most participants understood the information given in advance of the discussion quite well, as confirmed by the number of correct answers on the knowledge items of the short questionnaire. The small groups in our study led to an optimal involvement of the participants in the group-discussions and were good for the explorative purpose of this study.

However, this study has also some limitations. First, parents were discussing about a hypothetical question what they think of carrier disclosure after newborn screening while their child had not been born yet. Secondly, to get a broader opinion a larger number of parents, for example by using questionnaires should be included. Further, the participants in our focus groups were highly educated and mostly of Dutch origin. Therefore, these findings may not fully represent the point of view of the whole multi-ethnic population and their ability to understand this kind of health information.

In other studies with parents who already had a child who is a carrier, parents use similar arguments as the participants in our study.[12,13] Such as having the opportunity to test themselves to determine their risk for subsequent pregnancies, to make well-informed reproductive decisions, also with regards to future reproductive choices of their child, and to inform extended family members. Also the main argument of our participants for not wanting to be informed, no wish for further pregnancies, was

similar as in a previous study looking at the best moment for counseling of the parents.[4]

Earlier studies showed parental difficulties in informing family members.[14,15] The participants in our focus groups did not mention these problems, probably because they did not have the actual experience of informing their family.

The majority of participants strongly disagreed with the statements that carrier status should not be disclosed because carriers are healthy and not all carriers will be detected. They thought that parents should decide themselves what would be best for them and for their child. This was also the opinion of parents in another focus group study on newborn screening.[16] This may be an opt-in procedure in which parents indicate they want to be informed, or an opt-out procedure in which all parents are informed unless they indicate they do not want to know.

Health education of parents, or expecting parents, about newborn screening is very important. Parents need to make a well informed decision about the disclosure of a possible carrier result of their child. Therefore, midwifes or gynaecologists should preferably inform parents about newborn screening for CF and the possibility of carrier detection before the baby is born and a well informed professional should inform parents about a newborn carrier test result.

Conclusion

A majority of future parents prefer to be informed about the CF carrier status of their newborn because of implications for reproductive decisions for themselves, their child or extended family members, when a DNA-based newborn screening strategy for CF is implemented. However, parents point out that the choice of not being informed about the carrier status of their child should also be offered.

Acknowledegements

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CHAPTER 8

Differences in clinical condition after a diagnosis of cystic fibrosis by newborn screening or by symptoms

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Abstract

Background

Early diagnosis through newborn screening (NBS) and early treatment of cystic fibrosis (CF) does lead to better prognosis. In the Netherlands, the median age for a clinical diagnosis is six months, after newborn screening this is 30 days. It is unknown if the difference between being diagnosed at the age of five months or before two months is still clinically relevant.

Aim of the study

To assess the differences in clinical parameters at diagnosis between children with CF identified by newborn screening (NBS) or by clinical diagnosis (CD) in the Netherlands.

Methods

From July 1st, 2007 to January 1st, 2012 all newly diagnosed CF patients were reported to the Dutch Paediatric Surveillance Unit (DPSU). All paediatricians received a questionnaire to collect data on mutations and clinical condition at diagnosis. Non-classical CF was excluded from the analysis on clinical condition.

Results

204 new CF diagnosis were reported to the DPSU, 33 double and three had no CF after further testing. 127 questionnaires were returned (76%); 85 children were diagnosed because of clinical symptoms, 40 after NBS and two because of a positive family history. The median age at diagnosis was 34 weeks for a clinical diagnosis and 3 weeks after NBS. Compared to the NBS group, significantly more patients in the CD group showed failure to thrive, respiratory symptoms, and hospitalizations. 62% of the CD group showed abnormal signs at physical examination compared to 4% of the NBS group. Non-classical CF was more prevalent in the NBS group (6 clinical, 14 NBS), mostly F508del/R117H7T (12).

Conclusion

Infants detected after NBS are in a significantly better condition than after a clinical diagnosis, even when the difference in time at diagnosis between the two groups is only 5-6 months. Growth retardation is already seen when after NBS the diagnosis is confirmed, but NBS leads to a diagnosis before respiratory symptoms have developed.

Introduction

Newborn screening for cystic fibrosis (NBSCF) is being implemented in an increasing number of countries globally. The generally accepted opinion is that the benefits of newborn screening (NBS) outweigh the potential harm.[1]
Early diagnosis through newborn screening and early institution of treatment of CF does lead to better nutritional status, improved growth, longer preservation of lung function and longer survival in early adulthood.[2,3]
For maximum benefit of screening, the diagnosis should be confirmed before the age of two months.[4,5] In the 1990's the median age at diagnosis in the Netherlands was 14 to 18 months.[6] Recent data showed that the median age at diagnosis, excluding infants diagnosed through screening, was about five months in 2008.[7] At the same time the median age at diagnosis after newborn screening was 30 days, and all patients were diagnosed before the age of two months.[8] It is unknown if the difference between being diagnosed at the age of five months or before two months is still clinically relevant.
In this study we assessed the differences in clinical parameters at diagnosis between children with CF identified by NBS or by clinical diagnosis in the Netherlands.

Methods

Registration

From July 1, 2007 to January 1, 2012 all newly diagnosed CF patients in the Netherlands were registered by the Dutch Paediatric Surveillance Unit (DPSU). The DPSU was initiated by the Dutch Paediatric Society (NVK). The purpose of the surveillance system is to gain insight into the prevalence of rare and new diseases in children (0-18 year) on a population level, and to promote scientific research addressing the background, nature and prognosis, as well as the treatment and prevention, of these diseases.[9] Paediatricians were asked to report all new CF diagnoses monthly. Diagnosis was made by newborn screening, based on clinical symptoms or by family history. Confirmation of the diagnosis and treatment of CF is performed by seven CF centres in the Netherlands.

Newborn screening for CF

From January 1, 2008 until May 1, 2011, a pilot study of newborn screening on CF was performed in four provinces in the southern and middle part of the Netherlands (Cystic fibrosis Heel prick amOng a newborn Population In the Netherlands; CHOPIN study). In

the other areas, there was no newborn screening for CF.[8] From May 1, 2011 newborn screening for CF was added to the routine Dutch heel prick screening program.

Study population

All children diagnosed with CF, classic as well as non-classic CF in the Netherlands and reported to the DPSU between July 2007 and January 2012 were included in the study.

Classic and non-classic (equivocal) CF

Classic CF was defined as an infant with two cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations with symptoms and/or a positive sweat test result (sweat chloride value ≥60 mmol/l) or a positive family history.[10]
We defined non-classic CF as an infant with two CFTR mutations, of which one or two have an unclear phenotypic outcome and a normal or intermediate sweat test result (sweat chloride value between 30-60 mmol/l.[11]

Study design

We compared the prevalence and distribution of the registered CFTR mutations in both groups. We also compared the age and clinical condition at the time of diagnosis of children detected by NBS with children diagnosed clinically (CD).

Screening methods

During the pilot study two screening strategies were compared.[8] When newborn screening for CF was added to the Dutch routine newborn screening program, a four step approach, the IRT-PAP-DNA-sequencing strategy was used.[8] In this strategy, all screening samples with high concentrations of immunoreactive trypsinogen (IRT) as well as pancreatitis-associated protein (PAP) get a DNA-mutation analysis, followed by sequencing of the CFTR-gene when only one mutation is found. As a fail-safe procedure sequencing of the whole CFTR gene was also performed in all samples with an IRT ≥100 µg/l without identified mutations.
Mutation analysis was performed using the commercial kits INNO-LiPA CFTR19 and INNO-LiPA CFTR17+Tn. CFTR-mutation I148T was ignored because this mutation does not cause CF.
During the pilot study, infants with a positive screening test were referred to one of the four CF centres participating in the study and after the introduction of newborn screening for CF in the routine heel prick program to one of the seven CF centres in the Netherlands.

Questionnaires

The clinical condition was assessed by questionnaires sent to the attending paediatricians of the reported children. (see appendix)
In the questionnaires doctors were asked to record the following symptoms: meconium ileus, failure to thrive (growth below the 5th percentile or a change in growth that crossed two major growth percentiles in a short time), growth retardation and/or weight loss (Weight for height or weight below 50th percentile, physical growth significantly less than peers, or loss of weight), malabsorption, steattorhoea , recurrent airway infections (infection of the upper (cold) or lower airways (pneumonia) caused by viral or bacterial pathogens), chronic coughing (cough for more than 8 weeks), mucus production, dyspnoea, and ear- nose and throat (ENT) problems (nasal polyps, sinusitis, otitis).
The age at diagnosis, number of hospital admissions, the sweat test results, and the CFTR mutations were also recorded. If available, results of blood coagulation tests, vitamin A, D and E levels, chest X-ray, fecal fat (grams/24h), fecal elastase and sputum cultures (throat or cough swab) were also recorded.

Statistical methods

Results were considered significant if $p<0.05$. We used the Pearson chi-square test and the Fischer's exact test to compare the differences of symptoms, clinical signs at physical examination, and additional testing in the two groups. Non-parametric tests were used to compare median age at diagnosis and growth parameters (Mann-Whitney-U test).

Results

From July 2007 to January 2012, 204 children with CF were reported to the DPSU (17 children were registered in 2007, 45 in 2008, 65 in 2009, 38 in 2010 and 39 children in 2011). However, 33 children were reported twice and three children did not appear to have CF after further diagnostic testing. Therefore, a total number of 168 CF patients were recorded during the study period. The response rate of the questionnaires was 76% (127/168). The reported 24% patients of whom no questionnaire was returned, consisted of 23 CD and 18 NBS patients (Chi^2 p=0.273).
85 patients were found because of clinical symptoms (CD), 40 by newborn screening (NBS), and two were found by family history (see figure 8.1). Two infants with a diagnosis after a positive family history were excluded from the analysis on clinical symptoms and physical signs, because they were diagnosed early but not by NBS or symptoms.

Table 8.1 shows the median age at diagnosis of the patients in the two groups. The median age at diagnosis in the clinical diagnosis (CD) group of 34 weeks (31.5 weeks for classic CF) was significantly higher than the median age at diagnosis after newborn screening of 3.0 weeks (Mann-Whitney-U, p<0.001).

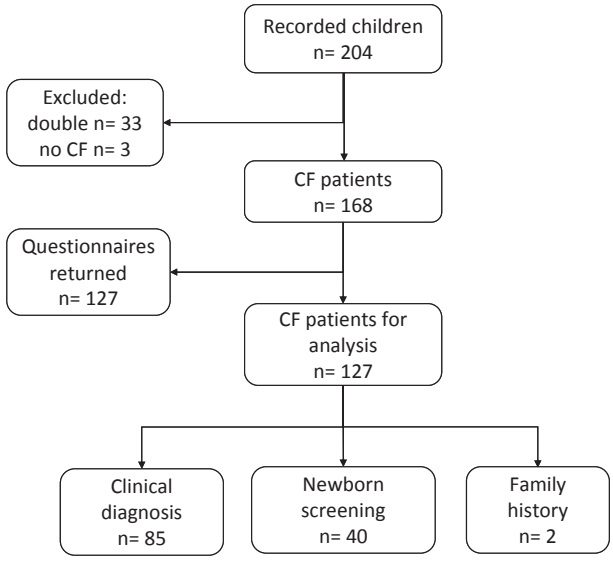

Figure 8.1 Study population.

Table 8.1 Median age at diagnosis for children with classical cystic fibrosis after a clinical diagnosis or detected after newborn screening. The difference is statistically significant (Mann-Whitney-U test, p<0.001).

Diagnosis	Median (weeks)	Interquartile range (weeks)
Clinical diagnosis (n=85)	34.0	10.0-113.0
Newborn screening (n=40)	3.0	3.0-5.0

Comparison of distribution and frequency of CFTR mutations between NBS and CD group

The DNA mutations were known for 82/85 of the children with a clinical diagnosis and all children identified by NBS (n=40) or a family history (n=2)(Table 8.2).

Table 8.2 CFTR mutations detected at DNA mutation analysis after a clinical diagnosis and newborn screening.

CFTR-mutations	CD-group n (%) n= 82	NBS-group n (%) n=40	Family history n (%) n=2	Total n(%) n=124
F508del/F508del	58 (71)	19 (48)	1 (50)	78 (63)
F508del/R117H-7T	1 (1)	11 (28)	0	12 (10)
F508del/A455E	4 (5)	2 (6)	0	6 (5)
F508del/S1251N	2 (2)	0 (0)	0	2 (2)
F508del/R1162X	1 (1)	1 (3)	0	2 (2)
F508del/E60X	2 (2)	0	0	2 (2)
F508del/G461R	2 (2)	0	0	2 (2)
F508del/L927P	2 (2)	0	0	2 (2)
F508del/G542X	1 (1)	1 (3)	0	2 (2)
F508del/G576A	0	0	1 (50)	1 (1)
F508del/E730X	0	1 (3)	0	1 (1)
F508del/3849+10KBC>T	1 (1)	0	0	1 (1)
F508del/2184delA	0	1 (3)	0	1 (1)
F508del/621+1G>T	1 (1)	0	0	1 (1)
F508del/Leu453Ser	1 (1)	0	0	1 (1)
F508del/G85E	0	1 (3)	0	1 (1)
F508del/711+1G>T	1 (1)	0	0	1 (1)
F508del/N1303K	1 (1)	0	0	1 (1)
F508del/1653delCTT (CFTR exon 10) and CFTR deletion exon 17a and 17b	1 (1)	0	0	1 (1)
F508del/'unknown'	1 (1)	0	0	1 (1)
R553X/R117H-7T	0	1 (3)	0	1 (1)
R553X/1789>T and 4243-3T>A	1 (1)	0	0	1 (1)
S1251N/R117H-7T	0	1 (3)	0	1 (1)
R1162X/R117H-7T	0	1 (3)	0	1 (1)
IVS-8 5T/ IVS-8 5T	1 (1)	0	0	1 (1)

CFTR= cystic fibrosis transmembrane conductance regulator, DNA= deoxyribonucleic acid, CD= children diagnosed clinically, NBS= children detected by newborn screening

Classic and non-classical cystic fibrosis

In the CD group 79 children had classic CF and 6 children were diagnosed with non-classic CF compared to 26 patients with classic CF and 14 with non-classic CF in the NBS group. Non-classical CF was significantly more prevalent in the NBS group ($p<0.001$).

CFTR-mutation frequencies

F508del was expected to be most common and this was confirmed in our registration (Table 8.2). The F508del counted for 212 of the 248 alleles (85%). The R117H-7T was the second most forthcoming mutation with a frequency of 15/248 (6%). R117H-7T was mostly seen in patients diagnosed by NBS and less prevalent after a clinical diagnosis (14 versus 1 respectively).

Homozygote versus heterozygote

In the CD group 71% (58/82) of the patients was homozygous for the F508del compared to 48% (19/40) in the NBS group (Table 8.2). In the CD group 27% (26/82) was heterozygous for F508del, in the NBS-group this was 54% (18/40). In the CD group, 2% (2/82) had two different CFTR-mutations compared to 8% (3/40) in the NBS group. Excluding non-classical CF led to a different result: CD 76% (58/76) homozygous F508del and 24% (18/76) heterozygous compared to respectively 73% (19/26) and 27% (7/26) for the screened group.

Symptoms at diagnosis

Because of a significant difference between the CD group and NBS group regarding the number of patients with non-classical CF, all analyses were carried out with and without the patients with non-classical CF (Table 8.3)
Meconium ileus was seen in 10% infants in the CD group and 17% of the NBS group (p=0.486). When comparing the patients with classic CF, 96% patients in the CD group had symptoms at diagnosis while in the NBS group this was 50%, independent if they had MI or not. Significantly more patients in the CD group compared to the NBS group had failure to thrive, steatorrhoea, recurrent airway infections, chronic coughing, mucus production , dyspnoea, and hospital admissions in the medical history. Children with non-classic CF showed symptoms in 100% of the CD group (growth reduction and respiratory symptoms) and 14% of the NBS group (reduced growth).

Table 8.3 Comparison of symptoms of CF at diagnosis after a clinical diagnosis and after newborn screening for classical CF.

	Clinical diagnosis n=79 %(n)	**Screening n=26 %(n)**	**p-value**
1 or more clinical symptoms (shown below)	96 (76)	50 (13)	<0.001*
Meconium ileus	10 (8)	17 (4)	0.486*
Failure to thrive	53 (42)	15 (4)	0.001*
Poor growth	47 (37)	27 (7)	0.108*
Malabsorption	38 (30)	23 (6)	0.234*
Steathorroea	44 (35)	8 (2)	0.001*
Airway infections	43 (34)	0 (0)	<0.001*
Coughing	33 (26)	0 (0)	<0.001*
Sputum	20 (16)	0 (0)	0.010*
Dyspnoea	29 (23)	4 (1)	0.007*
ENT problems	14 (11)	0 (0)	0.062*
Hospital admissions	70 (55)	23 (6)	<0.001*
SDS height (SD)	-1.25 (1.21)	-0.96 (1.45)	0.427†
SDS weight (SD)	-1.72 (1.27)	-1.14 (0.58)	0.068†
SDS head circumference (SD)	-1.05 (1.11)	-0.78 (1.04)	0.532†

*Fisher's exact test. †independent sample t-test. CF=cystic fibrosis, ENT=ear-nose-throat, SDS=standard deviation score, SD standard deviation

Signs at physical examination

Almost 62% of infants in the CD group had signs at physical examination, compared to 4% in the NBS group. Most children had signs of airway problems, such as dyspnoea, tachypnoea or abnormalities at auscultation of the lungs (Table 8.4). Infants with non-classical CF detected after NBS showed no abnormal signs at physical examination compared to two out of seven non-classical CD children with abnormal auscultation (p=0.091).

Table 8.4 Data on physical examination at diagnosis (classical CF).

	CD % (n) n=76	NBS % (n) n=25	p-value*
Signs at physical examination (1 or more)	62 (47)	4 (1)	<0.001
Abnormal auscultation†	46 (35)	0 (0)	<0.001
Dyspnoea and/or tachypnoea	43 (33)	4 (1)	<0.001
Digital clubbing	7 (6)	0 (0)	

*Fisher's exact test. †Abnormal auscultation is decreased breath sounds or presence of crackles or wheezing.
CF=cystic fibrosis, CD=clinical diagnosis, NBS=newborn screening

Results of additional biochemical tests

Unfortunately in only 13 of the infants of the NBS group the results of additional tests were available (Table 8.5).
In the CD group 87% (29/33) was pancreas-insufficient, fecal fat was increased in 87% and elastase in 92%, in the NBS group 75% (6/8) of the CF infants was pancreas-insufficient. Vitamin deficiency was present in about 23% of the CD group, low levels for vitamin A and E were already seen in NBS infants as well as a prolonged coagulation time (Table 8.5). Children with non-classical CF showed no significant differences between CD and NBS group.

Radiology findings

Chest X-rays were performed in 51 children of the CD group and nine of the NBS group. Chest X-rays showed moderate signs of lower airway involvement (airtrapping, atelectasis, infiltrates) in 17 children of the CD group and 21 had severe findings (bronchiectasis). In only nine infants of the NBS group a chest X-ray was performed, in this group one infant had moderate and another infants had severe signs on the chest X-ray at diagnosis. HRCT's were not yet performed in newborns after NBS.

Table 8.5 Laboratory findings for children with classic CF at diagnosis based on clinical symptoms or newborn screening.

	CD (n/total)	NBS (n/total)	p-value*
Elastase < 500 µg/g	22/24	6/6	0.562
Fecal fat > 3 gram/24 hours	20/23	1/3	0.085
Prolonged coagulation time (APTT and PT)	6/58	4/12	0.061
Vitamin A decreased	12/64	6/11	0.019
Vitamin D decreased	2/19	0/1	
Vitamin E decreased	15/65	1/11	0.440

Cut-off levels for elastase: normal above 200 µg/g, Fecal fat normal < 3 gram/24 hours, APTT normal 20-45 sec, PT normal 11-14 sec, Vitamin A cut-off levels: 1-6 years 0.7-1.5, 7-12 years 0.9-1.7, > 12 years 0.9-2.5 µmol/l, Vitamin D: 30-130 nmol/l, Vitamin E newborn: 5-9, 1-6 years: 10-21, 6-19 years: 13-24 µmol/l. *p-value determined by Chi-square or Fischer's exact test. CF=cystic fibrosis, CD=clinical diagnosis, NBS=newborn screening, APTT= Activated Partial Thromboplastin Time, PT= Prothrombin time

Results of microbial cultures

Not all children were cultured at diagnosis (n=74/81 CD and 19/26 NBS). In the children with classic CF, about 74% of the CD group and 53% of the NBS group carried one or more microbial pathogens in their throat or sputum. Pseudomonas aeruginosa was not found in the NBS group, but was seen in 7% of the CD group at diagnosis. In 25% of the NBS group Staphylococcus aureus was found, compared to 45% in the CDgroup.

Discussion

After NBS the median age at diagnosis is three weeks, and all infants were diagnosed before the age of two months, while with a clinical diagnosis this was six months. It is unlikely that a clinical diagnosis can be made earlier than found in this study. The pilot study for NBS took place during the period of registration and most probably led to a high awareness for CF and symptoms of the disease among paediatricians, but the age at clinical diagnosis did not change.

The genetic background of the NBS group differed significantly from the CD group with 14 (35%) patients with non-classical CF against six (7%) in the CD group, mostly due to R117H-7T as the second mutation.

Not unexpectedly, children with a classic CF diagnosed because of newborn screening showed significantly less symptoms at the time of diagnosis than children detected by clinical symptoms. However, we found that a third of infants with CF detected by NBS already had signs of malabsorption or poor growth at the age of three weeks. Respiratory signs or symptoms were present in about 43% of the children in the CD

group compared to only 4% in the NBS group. P. aeruginosa was not cultured in the NBS group, but a fourth already carried S. aureus. In 70% of the CD group one or more hospital admissions preceded the diagnosis.

Many studies have compared long term prognosis after a diagnosis by NBS or clinically, but very few looked at the clinical condition at the time of diagnosis. A historical cohort study showed that children had significantly lower z-scores for height and weight at diagnosis, similar to our results.[12] We found that, while growth retardation occurs very rapidly in infants with CF, respiratory involvement develops more slowly. Infants in the CD group had significantly more respiratory signs at physical examination, compared to only one infant in the NBS group with abnormal auscultation of the lungs. Chest X-rays showed pulmonary infection, atelectasis and bronchiectasis in the CD group, but showed abnormal signs in only a small percentage in the NBS group. The Wales/West Midlands study showed no differences between a NBS and CD cohort.[13] In the Wisconsin study the CD group had worse chest X-ray findings at diagnosis than the NBS group.[14] A Dutch study showed similar results.[15] However, in none of these studies clinical evaluation was performed immediately after a diagnosis by NBS but at a later age.

To our knowledge, this is the first study showing that at the age of three weeks only very few infants with CF have respiratory disease. This can explain the observation that patients with CF maximally benefit from a confirmed diagnosis before the age of two months.[4] Our findings show that NBS offers a window of opportunity to start treatment before pulmonary disease has arisen. An Australian study showed that lung function measured by forced expiration is normal in infants at the time of diagnosis (6 weeks to 30 months) but diminished in older infants, suggesting that in CF the optimal time for therapeutical interventions for preservation of lung function may be within the first six months of life.[16] NBS also provides the opportunity to diagnose patients before pseudomonas colonization and to prevent or postpone chronic colonization.[17,18] Although the Wisconsin study showed that in the NBS group pseudomonas was found more often, this was caused by one centre that did not perform segregation of patients which was probably the cause of earlier acquisition of pseudomonas.[14] Several studies showed a longer median time to acquisition of pseudomonas after NBS.[2,15,19]

The F508del mutation is the most prevalent in the Netherlands, similar for NBS and CD. R117H-7T as second mutation is more frequently seen after NBS and rarely at a clinical diagnosis. A French study showed similar results.[20] Detection of mild mutations by NBS leading to non-classical CF (equivocal CF diagnosis) has led to a not yet resolved debate, because most infants will never develop any symptoms or only mild pulmonary symptoms in adulthood and probably do not benefit from detection by NBS. Therefore, some countries exclude the R117H-7T mutation from the screening panel.[21]

Strengths and weaknesses of this study

After the start of NBS for CF regionally and from May 2011 nationwide we were able to prospectively compare children diagnosed through NBS or by clinical symptoms, born in the same time and the same country.

The two groups of CF patients in this study differed significantly with proportionally more non-classical CF in the NBS group. Inclusion of children with non-classical CF will lead to more favourable results for NBS. To avoid this problem in the analysis of clinical disease, we did all analyses separately for classic and non-classical CF. We did not receive questionnaires of 24% of reported children, this group consisted of relatively more screened infants than clinical diagnosis, this may have influenced our data however the difference between groups was not significant.

Unfortunately not all biochemical tests and chest X-rays were performed in all infants. The results should therefore be interpreted cautiously, as selection bias cannot be excluded.

The problem of using questionnaires is that the various items may be interpreted differently by the paediatric pulmonologists. On the other hand, in the Netherlands all CF centres use the same guidelines for diagnostic tests and treatment of children and adults with CF, therefore it is unlikely that differences between CF centres and doctors have influenced our results to a great extent.[22]

Participation of paediatricians in the DPSU is high, about 93%.[9] We included 127 of 168 registered CF diagnoses, which probably is a representative number.

Conclusion

Infants detected by NBS are in a significantly better clinical condition than CF children with a clinical diagnosis, even when the median age at clinical diagnosis is relatively young. Growth retardation is already seen when after NBS the diagnosis is confirmed, but in most cases NBS leads to a diagnosis before respiratory symptoms of CF have developed. This is probably critical for achieving an optimal outcome.[2,4]

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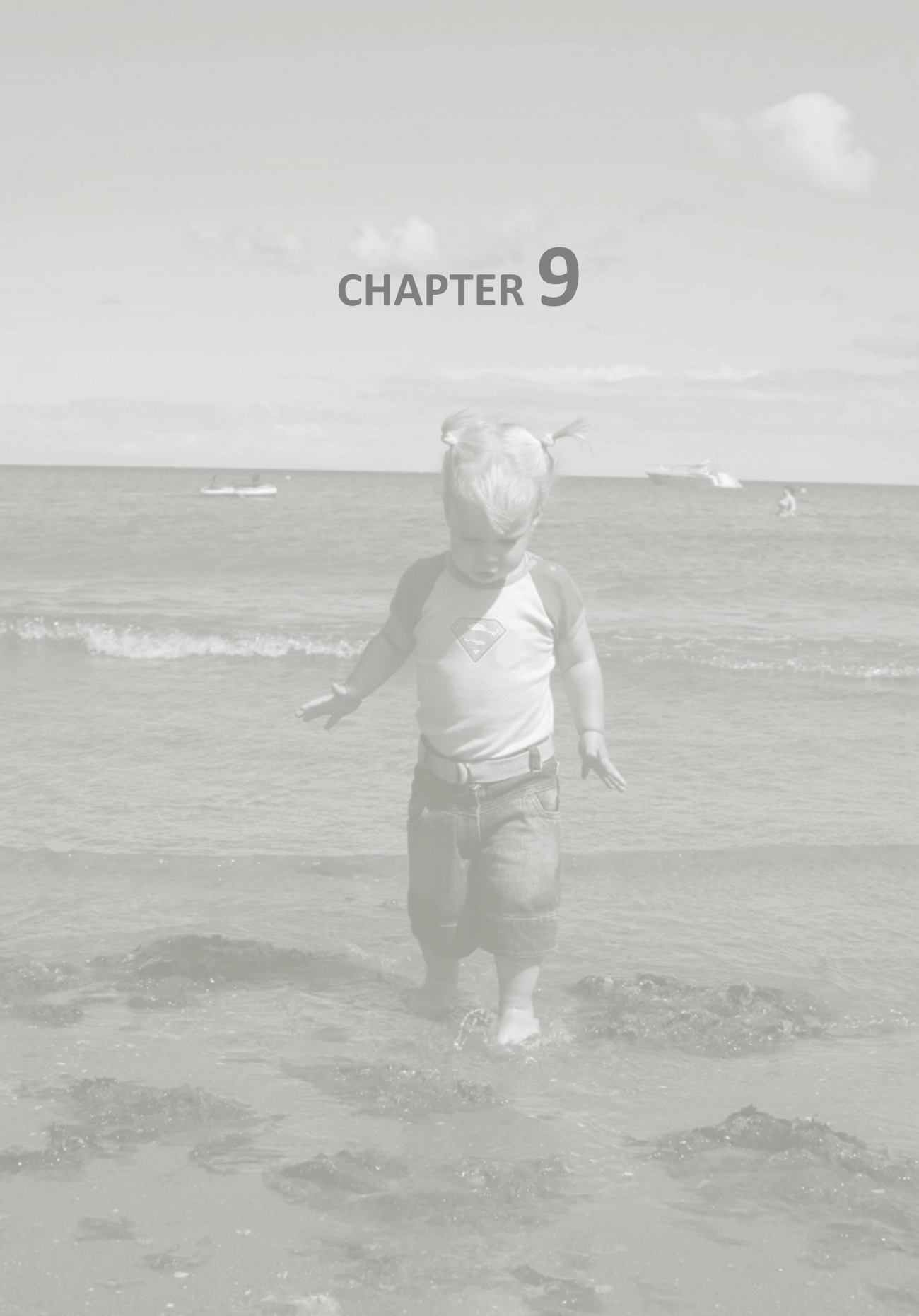
CHAPTER 9



Clinical evaluation of the Nanoduct sweat test system in the diagnosis of cystic fibrosis after newborn screening

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Abstract

Background

After a positive newborn screening test for cystic fibrosis (CF), a sweat test is performed to confirm the diagnosis. The mean estimated success rate of the generally acknowledged methods (Macroduct/QPIT) in newborns is 87%. The Nanoduct sweat test system is easier to perform and less sweat is needed.

Objectives

To investigate the success rate of the Nanoduct in newborns compared to the Macroduct/ QPIT, and to explore the optimum cut-off points for (exclusion of) a CF diagnosis.

Methods

After informed consent of the parents, newborns with a positive screening result for CF were included. The Macroduct/QPIT and Nanoduct were performed in all infants, during the same appointment. Sweat production and collection were performed according to international guidelines. Chloride concentration was determined by standard colorimetry, conductivity was measured directly and converted to a NaCl molarity.

Results

108 newborns were included: 17 with CF, 7 non-classical CF, and 84 non-CF children. The success rate of 93% for the Nanoduct was significantly better than that of 79% for the Macroduct/QPIT (McNemar, p=0.002). The Nanoduct detected the same CF patients as the Macroduct/QPIT, one CF patient had an equivocal result for both tests, and no patients were missed. The area under the Reciever Operating Characteristic-curve for detection of CF with the Nanoduct was 0.999, with ideal cut-off levels 91 mmol/l and 66 mmol/l.

Conclusion

In newborns, the success rate of the Nanoduct is significantly higher than for the Macroduct/QPIT. The Nanoduct seems a reliable method to confirm or exclude the diagnosis CF after newborn screening. The ideal cut-off points for conductivity in our population were comparable to former studies.

Introduction

Cystic fibrosis (CF) is one of the most common inherited diseases in Caucasian populations, with around one in 1,500 to 5,000 newborn infants affected.[1] CF is a multi-organ disease involving the airways, pancreas, liver, intestine, sweat glands, and vasa deferentia. Newborn screening for CF (NBSCF) leads to an early diagnosis and early treatment, and is accompanied by a better prognosis.

NBSCF is implemented in more and more countries over the world. The positive predictive value (PPV) of most screening programs is 12 to 25%, which means many false-positive results. The period between the test result and the diagnosis is very stressful for the parents.[2,3] The diagnosis CF should be made before the age of two months to have a maximal benefit from an early diagnosis.[4] Therefore, the period between the positive screening test and the actual diagnosis should be as brief as possible. In newborn screening programs for CF, a reliable and fast diagnostic test is vital to discriminate between CF patients and healthy baby's. The gold standard test to confirm or exclude the diagnosis CF after newborn screening is determination of the sweat chloride concentration by a sweat test.[5]

The quantitative pilocarpine ionthophoresis test (QPIT) and the Macroduct collection system are internationally accepted methods for sweat collection.[6] However, with these methods it is difficult to collect sufficient sweat samples in infants at the age of 3-4 weeks. The failure rate of the Macroduct varies from 3.5 to 26.2%,[7-11] and the QPIT yields insufficient samples in 0.7 to 14.3% of term infants.[7,8,10,11] The failure rate is higher in preterm infants and infants less than 6 weeks postnatal age.[12,13] Skin color or race may also lead to higher failure rates.[12,14] The Nanoduct was especially designed for newborns and may offer a better opportunity in the follow-up of infants with a positive screening test. Previous studies showed a success rate of 90.9% for the Nanoduct,[9,15] but all studies included populations of various ages which makes it difficult to generalize the results to a population of newborns.

We investigated if the Nanoduct sweat test system is successful and feasible in newborns with a positive newborn screening test for CF in comparison with the gold standard (QPIT/Macroduct), and assessed the cut-off values for diagnosis or exclusion of CF.

Methods

Study design

This study was a prospective comparative diagnostic test accuracy study in a paired study design and part of a large study on novel strategies in newborn screening for CF

in the Netherlands, the Cystic fibrosis Heel prick amOng a newborn Population In the Netherlands (CHOPIN)-study.[16] Newborns were screened with two strategies: The first strategy consisted of immunoreactive trypsinogen (IRT) followed by pancreatitis-associated protein (PAP) assessment in heel prick blood. In the second strategy, a high IRT was followed by DNA mutation analysis (35 mutations) and sequencing when a single mutation was found. All newborns with a positive screening test were referred to a CF centre for a sweat test to confirm or exclude the CF diagnosis. The CF centres received information about the CFTR mutations of the referred infants only after performing the sweat tests.

The Nanoduct was compared with the QPIT or the Macroduct and both tests were carried out at the same appointment. Only the sweat tests performed during the first appointment upon referral were included in this study .

All CF-centers participating in the CHOPIN study cooperated; the Maastricht University Medical Center (MUMC), the University Medical Center Nijmegen (UMCN), the Erasmus Medical Center (EMC), and the University Medical Center Utrecht (UMCU). The participating CF-centers got referred infants depending on their region of referral as determined for NBS. Two centres (UMCU and EMC) used the QPIT, and the other two centers (MUMC and UMCN) used the Macroduct.

Study population

Between 1-1-2008 and 1-1-2012 all newborns <2 months of age referred to the hospital for a sweat test after a positive newborn screening test result were included after informed consent and permission of the parents. Newborns with severe eczema or sepsis were excluded. In premature infants (<37 weeks) the sweat test was performed at the term age (≥38 weeks).

Definition of CF and equivocal diagnosis

The QPIT and Macroduct were used as reference test. The diagnosis of CF was confirmed by a sweat chloride concentration of ≥60 mmol/l. If this was not possible or the sweat test failed, the diagnosis could also be confirmed by two CFTR mutations, and/or a meconium ileus and/or positive family history.[5,17]

An equivocal diagnosis was defined according to international guidelines as an equivocal sweat test result (chloride 30-60 mmol/l) or a normal sweat test (chloride under 30 mmol/l) on two occasions in a newborn with two CFTR mutations of which one or both have an unclear clinical significance.[17]

Sweat test methods

International guidelines for the performance of sweat tests were followed.[18] Trained, skilled, and experienced personnel performed the test.

QPIT

After cleaning the selected area of skin, the electrolytes with Pilogel were placed on the infant's arm. The Webster (Elitech group Wescor Biomedicals, South Logan, USA) sweat inducer (model 3700) was used for iontophoresis (5 minutes at 1.5 mA). Once iontophoresis was complete, the electrolytes were removed and the skin underneath was cleaned with distilled water. Sodium free filter paper (e.g. Whatman no 41/42/44/541Whatman Nederland BV, 's Hertogenbosch, the Netherlands) was placed over the stimulated area and immediately covered with parafilm or polyethylene and sealed with tape. After 30 minutes the filter paper was taken off and weighed in a transport bottle. The minimum acceptable weight of sweat (75 mg) is corresponding with a sweat rate of 1 g/(m^2 x min); [10 x weight (mg/μl)/ collection area (cm^2) x time (min)]. The sweat produced was eluted from the filter paper into a suitable diluent and analyzed for chloride. Sweat chloride (and Sodium) was measured by a standard colorimetric procedure.

Macroduct

The Macroduct (Elitech group Wescor biomedical, South Logan, USA) was applied. After cleaning the selected area of skin, the electrolytes with Pilogel were placed on the infant's arm. The Webster sweat inducer (model 3700) was used for iontophoresis (5 minutes at 1.5 mA). Once iontophoresis was complete, the electrolytes were removed and the skin underneath was cleaned with distilled water. The Macroduct Sweat collector (Wescor) was attached to the stimulated skin. When collection was completed after a maximum of 30 minutes, the spiral tubing containing the sweat sample was extended and severed from the base. The minimum sweat rate needed was 1 g/(m^2 x min), this corresponded with a minimum amount of 15 μl. Sweat chloride (and sodium) was measured by a standard colorimetric procedure.

Nanoduct

The Nanoduct® Sweat (Elitech group Wescor biomedicals, South logan, USA) was used. The skin on the test site was cleaned and pilogel pads were placed on the infant's arm (or leg). Pilocarpine ionthophoresis was used to stimulate sweat production; this took 2-3 minutes (at 0.5 mA). Conductivity was measured by continuous flow sensometry and required a minimum sweat rate of 1 g/(m^2 x min). The sweat conductivity was measured during the sweat collection. The cut-off points for the diagnosis CF by means of conductivity measurement are not completely clear, but most studies use 80-90 mmol/l.[8,9,11,15,19,20] In our study we used cut-off levels of 60 and 90 mmol/l to

exclude or confirm the diagnosis of CF, and conductivity was considered equivocal between 60 and 90 mmol/l.

Risk factors and side-effects

To determine possible risk factors for failure of the sweat test, we documented gestational age, birth weight, recent weight and length, and skin color on a form. We also registered side-effects as redness or blistering of the skin, and crying during the test. Forms were filled in by the performer of the sweat test.

Statistics

The Nanoduct was compared with the gold standard (QPIT or the Macroduct). Both are recommended and internationally accepted sweat test methods.[18] The success rate of the Macroduct/QPIT, taken together, was estimated to be about 80%.[7,8] The success rate of the Nanoduct versus the gold standard sweat test was used as outcome parameter. We expected that the Nanoduct would fail in 10% of the babies.[9,15] We expressed the success rate of the three tests as a percentage. Statistical analysis was performed with McNemar's test. We calculated that with an α=0.05 and a power (1-β) = 0.80, 100 babies have to be included to find this difference. The sensitivity, specificity and positive predictive value of the Nanoduct were calculated and compared with the gold standard tests. Receiver operating Characteristic (ROC) curves were established with measurement of the area under the curve (AUC) to determine the accuracy of the Nanoduct and to investigate the effect of different cut-off points for sensitivity and specificity. The data of risk factors were observational. Correlations between risk factors and success of the sweat test were calculated with Pearson correlation coefficients for continuous variables and Spearman correlation coefficients for non-parametric variables.

Results

Population data

Between 1-1-2008 and 1-1-2012, 105 of 179 infants with a positive screening test for CF could be included in this study. Another three infants were included after nationwide implementation of NBSCF in the Netherlands (IRT-PAP-DNA-sequencing). Three parents refused participation, five infants were not sweat tested for several reasons (died soon after birth because of congenital problems (n=2), clinical diagnosis with a positive family history and two mutations(n=3)). Five infants were excluded because they were sweat tested after the age of two months, because of prematurity (n=2) or they were included at their second sweat test appointment (n=3). In the other

61 infants the Nanoduct was not performed for operational reasons: the test could not be performed because of delayed delivery of the gel pads, or not enough personnel to perform both tests. The QPIT was performed in 18 infants and the Macroduct in 90 infants, the Nanoduct was performed in 108 infants (Figure 9.1).

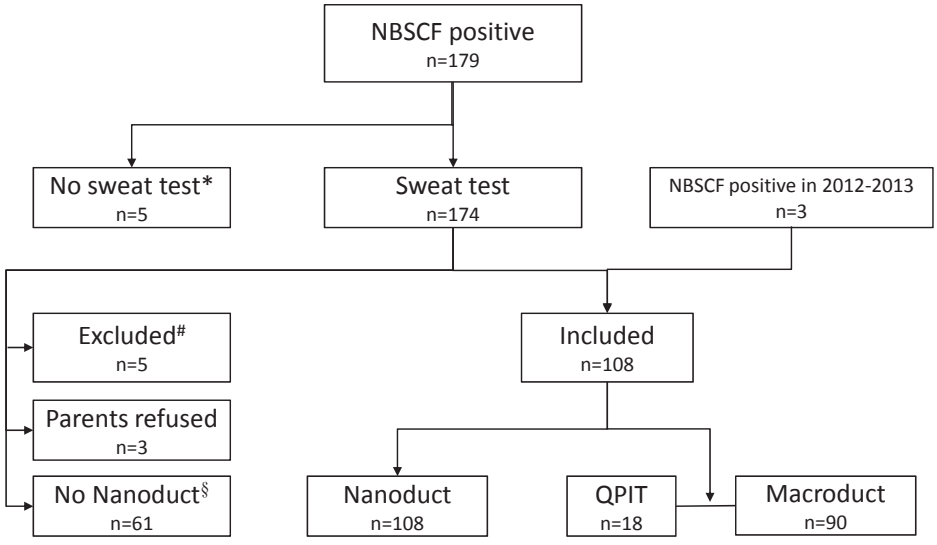

Figure 9.1 Flowchart infants included in the Nanoduct study.
*No sweat test: died soon after birth because of congenital problems (n=2), clinical diagnosis with a positive family history and two mutations(n=3). #Excluded because sweat performed after the age of two months, because of prematurity (n=2) or they were included at their second sweat test appointment (n=3). §No Nanoduct performed for several reasons: delayed delivery of the gel pads, or not enough personnel to perform both tests. NBSCF=newborn screening for cystic fibrosis, QPIT=quantitative pilocarpine ionthophoresis test.

Success rates

The QPIT/Macroduct failed in 23 infants, the success rate was 79% (85/108), (QPIT 83% (15/18) and Macroduct 78% (70/90)), the Nanoduct was successful in 92.5% (101/108). The success rate of the Nanoduct was significantly higher compared to the Macroduct/QPIT (difference 13.5%, McNemar p=0.002)

Diagnosis of CF

In the study population of 108 infants, CF was diagnosed in 17 infants, excluded in 84 infants, and seven infants had non-classic CF. Population characteristics are shown in Table 9.1.

Table 9.1 The population characteristics of the 108 infants with a positive newborn screening test included in the Nanoduct study. No statistical significant differences were found between the three groups.

	No CF (n=84)	CF (n=17)	non-classic CF (n=7)
Gestational age (weeks)	39.5 (2.2; 26.3-42.0)	38.8 (1.4; 36.4-41.0)	39.1 (1.2; 37.8-41.0)
Birth weight (gram)	3367 (595; 760-4540)	3004 (267; 2575-3615)	3323 (651; 2545-4140)
Age at sweat test (days)	30.1 (12.3; 17-90)	27.8 (10.5; 12-61)	34.4 (13.7; 24-63)

Data are mean (SD; range). CF=cystic fibrosis, non-classical CF=infants with one or two mutations with an unclear clinical significance and a normal or equivocal sweat test

Table 9.2 shows the results for the QPIT/Macroduct (chloride concentration) and Nanoduct (conductivity) for infants with CF, non-classical CF and no CF according to the cut-off levels. For the Macroduct/QPIT, the diagnosis CF was confirmed in 8 infants, and excluded in 69 infants. The QPIT/Macroduct failed in 23 infants, of which 7 had CF and one non-classical CF. Five infants had an equivocal sweat test result; one of them had two CF-causing mutations and four had no mutations in the screening test. Six infants had non-classical CF and a normal sweat test. The Nanoduct showed conductivity levels above 90 mmol/l in 15 of 17 infants with two CF-causing mutations; one had an equivocal conductivity result (conductivity 60-90 mmol/l) and one CF-infant had an insufficient sweat sample. All infants with non-classical CF had conductivities below 60 mmol/l. Both test methods succeeded in 74 of 108 infants. In this group the Nanoduct detected the same CF patients, one infant with CF had an equivocal result for both methods and no patients were missed.

Table 9.2 Chloride concentration measured by the Macroduct/QPIT and conductivity measured by the Nanoduct for infants with CF, non-classic CF and no CF according to their cut-off levels.

Chloride concentration* (mmol/l)	CF	Non-classic CF	No CF
failed	7	1	16
<30	0	6	64
30-60	1	0	4
≥60	9	0	0
Total	17	7	84

Conductivity† (mmol/l)	CF	Non-classic CF	No CF
failed	1	0	5
< 60	0	7	75
60-90	1	0	4
≥ 90	15	0	0
Total	17	7	84

*CF confirmed when chloride concentration ≥ 60 mmol/l, excluded when < 30 mmol/l, between 30 and 60 mmol/l the result is equivocal and the test should be repeated. †CF confirmed when chloride concentration ≥ 90 mmol/l, excluded when < 60 mmol/l, between 60 and 90 mmol/l the result is equivocal and the test should be repeated. The sweat test failed when the sweat rate was too low and no sufficient sweat sample could be obtained.

CF=cystic fibrosis, non-classical CF=infants with one or two mutations with an unclear clinical significance and a normal or equivocal sweat test.
The Nanoduct discriminated significantly between CF and no CF or non-classic CF ($p<0.001$), using the internationally recommended cut-off levels of 60 and 90 mmol/l (Figure 9.2). The difference between no CF and non-classical CF could not be made by any of the three methods.

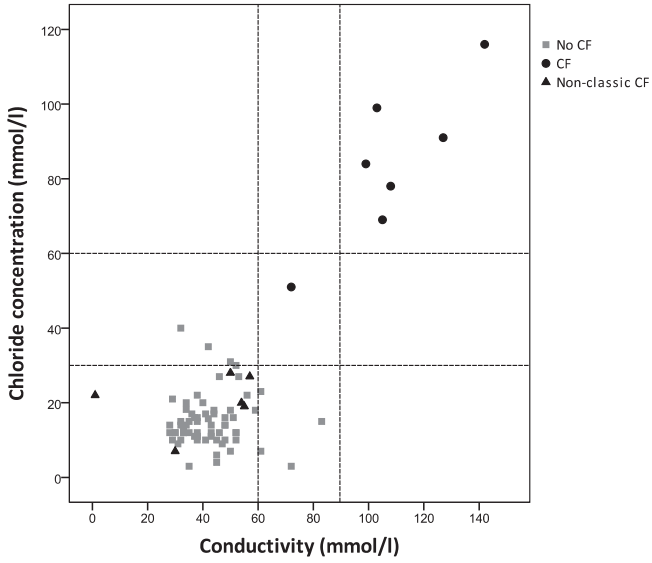

Figure 9.2 Correlation between chloride concentration measured by Macroduct/QPIT and conductivity determined by the Nanoduct.
Cut-off levels are indicated in the figure, cut-off levels for conductivity (Nanoduct) were 60 and 90 mmol/l and for the chloride concentration (QPIT/Macroduct) 30 and 60 mmol/l. CF=cystic fibrosis, non-classical CF=infants with one or two mutations with an unclear clinical significance and a normal or equivocal sweat test.

Chloride concentration and conductivity

Table 9.3 shows the results for chloride concentration measured with the Macroduct/QPIT and conductivity determined by the Nanoduct sweat test system. The mean chloride concentration and the conductivity in CF infants were significantly higher than in non-classic CF and healthy infants ($p<0.001$, t-test)
The correlation between the chloride concentration and conductivity was good with a (Pearson-)correlation coefficient of 0.842 (Figure 9.3) . The mean difference between the chloride concentration (Macroduct/QPIT) and conductivity (Nanoduct) was 26.5 mmol/l (SD 13.2).

Table 9.3 Chloride concentration and conductivity for infants with CF, Non-classical CF and no CF.

	CF	Non-classical CF	no CF
Chloride concentration (mmol/l)	84.0 (21.0; 51-116)	20.5 (7.6; 7-28)	14.9 (7.0; 3-40)
Conductivity (mmol/l)	108.0 (22.1; 72-142)	41.2 (22.0; 1-57)	42.4 (10.6; 26-83)
mean difference	24.0 (11.7; 4-36)	20.7 (21.2; -21 - 36)	27.3 (12.5; -8 - 69)

Data are mean (SD; range). CF=cystic fibrosis, non-classical CF=infants with one or two mutations with an unclear clinical significance and a normal or equivocal sweat test.

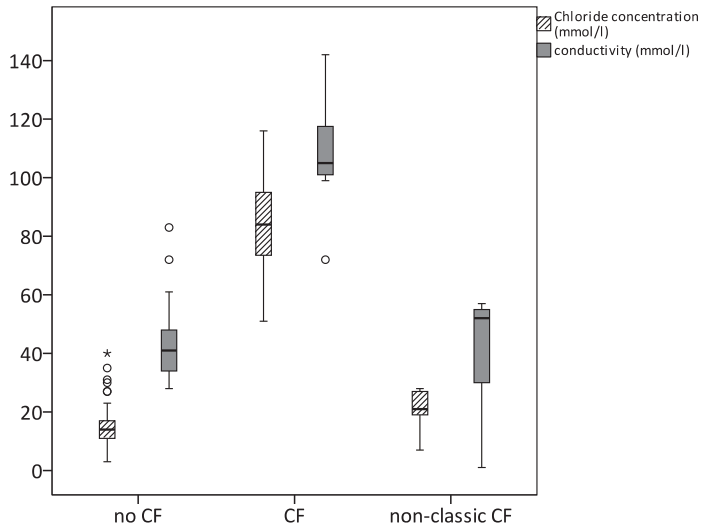

Figure 9.3 Chloride concentration and conductivity for different subgroups; CF, non-classical CF and no CF.
Chloride concentration (mmol/l) measured by the Macroduct/QPIT and conductivity (mmol/l) determined by the Nanoduct sweat test system. CF=cystic fibrosis, non-classical CF=infants with one or two mutations with an unclear clinical significance and a normal or equivocal sweat test.

Cut off levels

Former studies proposed cut-off levels of 60 and 90 mmol/l. When using those criteria the Nanoduct had a sensitivity of 100% (95% confidence interval (CI) 76-100) for the cut-off level of 60 mmol/l, 94% (CI 68-100) for the cut-off level of 90 mmol/l, and a specificity of 100% (CI 94-100) in our population (excluding infants with non-classic CF, who all had normal values). One infant with CF had an equivocal sweat test for the

Nanoduct and Macroduct/QPIT. In this study population, the sensitivity of the Macroduct/QPIT was 100% (CI 65-100) for the cut-off level of 30 mmol/l, 90% for 60 mmol/l and the specificity was 100% (CI 93-100).
The ROC curve showed an area under the curve of 0.999 (Figure 9.4). The best cut off levels to confirm or exclude CF were a conductivity of 66 mmol/l (sensitivity 100% (CI 76-100), and specificity 97.7% (CI 90.3-99.6)) and of 91 mmol/l (sensitivity 93% (CI 68-100) and specificity 100% (CI 94-100)). Under 66 mmol/l, no infant with CF would be missed and almost all infants with CF had conductivity levels above 90 mmol/l, between 60 and 90 the test should be considered equivocal.

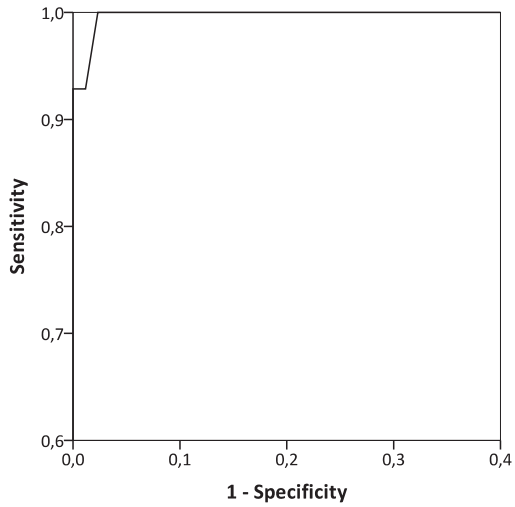

Figure 9.4 ROC curve for conductivity as a diagnostic test for CF.
ROC-curve for conductivity measured with the Nanoudct sweat test system to confirm the diagnosis cystic fibrosis, AUC was 0.999 with optimum cut-off levels to exclude CF under 66 mmol/l and for confirmation above 91 mmol/l. CF=cystic fibrosis, ROC=receiver operating characteristic, AUC=area under the curve.

Risk factors for failure of the sweat test

Gestational age of more than 37 weeks was significantly associated with a higher success rate for both methods (Spearman p=0.021 Macroduct/QPIT, p=0.048 Nanoduct). Birth weight, sex, age at sweat test and skin colour were not significantly correlated with the chance of success of the sweat test (QPIT/Macroduct or Nanoduct) (Table 9.4).

Table 9.4 The success rate of the QPIT/Macroduct and Nanoduct in relation to different population characteristics.

	QPIT/Macroduct % (n/total)	Nanoduct % (n/total)
Birth weight (gram)		
<2000	50 (1/2)	100 (2/2)
2000-4000	78 (70/89)	95 (84/88)
≥4000	83 (10/12)	92 (11/12)
Gestational age (weeks)		
<37	50 (5/10)	80 (8/10)
≥37	82 (77/94)	95 (89/94)
Sex		
Male	79 (41/52)	96 (49/51)
Female	78 (42/54)	93 (50/54)
Age at the sweat test		
<4 weeks	76 (45/59)	98 (57/58)
≥4 weeks	83 (38/46)	91 (42/46)
Skin color		
Black	67 (2/3)	100 (3/3)
Colored	100 (7/7)	100 (7/7)
White	86 (32/37)	89 (33/37)

QPIT=quantitative pilocarpin ionthophoresis test

Side effects

Redness of the skin was seen in 79% of infants after the Macroduct/QPIT, compared to 83% for the Nanoduct. Blistering of the skin did not happen. During the Nanoduct infants did not cry more than when the QPIT/Macroduct was performed, 15% and 23% respectively.

Discussion

We investigated the success rate and the reliability of the Nanoduct as a diagnostic procedure after a positive newborn screening result for CF in the Netherlands and found a significantly higher success rate for the Nanoduct than for the conventional methods (QPIT/Macroduct). The Nanoduct discriminated between CF and no CF and detected the same CF patients as the gold standard tests.

The Nanoduct has been studied in population of different ages, with success rates varying between 90.9% and 97.3%.[9,15,21] The largest study (n=1041) showed a success rate of 90.9%, most failures were in infants less than 1 month old.[15] The success rate was 93.7% in a subgroup of 237 infants less than 1 month old and 66 newborn and

preterm babies, comparable to our findings. Although the group in our study was very small, the Nanoduct also performed better in premature infants than the Macroduct/QPIT even when tested at a term age.

The cut-off point for the diagnosis CF by means of conductivity measurement is unclear, but most studies use 80-90 mmol/l.[9,11,15] It is also not clear which cut-off point should be used to exclude CF. The US Guidelines recommend that all individuals having a sweat conductivity ≥50 mmol/l should be referred to a CF care center for a quantitative sweat chloride test. A recent study advised under 50 mmol/l as normal and above 60 mmol/l as abnormal.[22] Our data suggest that after a positive newborn screening test CF can be excluded when the child shows no clinical symptoms and the conductivity is under 60 mmol/l. The diagnosis of CF can be confirmed with conductivity levels above 90 mmol/l.

Strength and weaknesses of our study

We calculated the number of infants needed for a significant effect on the success rate of the Nanoduct, and were able to include a sufficient number of infants for the aim of our study. The reference test (Nanoduct) and index test (Macroduct/QPIT) were performed in all included newborns and evaluated independently. The sweat test was performed before the DNA results were known by the paediatrician. Failure of sweat test was reported for both methods. The prevalence of CF in our population referred after positive NBS was very high. The prevalence of classic CF was 1:6 of referred infants, and 1:12 infants had non-classic CF. The positive predictive value of our IRT-PAP-DNA-sequencing program is 87%.

Unfortunately, 61 infants could not be included. During several months, there was an insufficient supply of electrodes to one centre and lack of personnel in another. We do not think this has led to selection bias because these problems occurred randomly and the population of 61 patients was not different from the whole population with respect to age, weight, sex, and percentage of CF.

According to the STARD-checklist used for diagnostic test accuracy studies, the Nanoduct looks feasible and reliable to detect CF in this population.[23]

The QPIT performed better than the Macroduct with success rates of 98% and 78% respectively. The QPIT was performed in only 18 infants and the Macroduct in 89, which made the groups too small for comparison with the Nanoduct separately. It might well be possible that the QPIT and Nanoduct have a comparable success rate, but even then the Nanoduct is much easier to perform.

US Guidelines advise the QPIT and Macroduct as the Gold standard test for CF diagnosis. Disadvantages are the duration of the test, the need for adequately trained and skilled personnel, and the minimum amount of tests needed to maintain good quality. The complexity of the current gold standard underlines the need for a new test

that can replace the existing gold standard. The Nanoduct has several advantages compared to the gold standard tests, such as a higher success rate, easier to accomplish, and immediately available test result. In a situation of highly alarmed parents due to the positive screening test, the Nanoduct can reassure much more rapidly than the gold standard tests. We, as others,[9,15,24] found that compared to the QPIT/Macroduct, the Nanoduct detects the same patients and healthy infants as the gold standard test. Therefore, the Nanoduct seems a good candidate for replacing the existing gold standard.

Using the internationally recommended cut-off levels of 60 and 90 mmol/l for conductivity, the sensitivity and specificity of the Nanoduct are comparable to the Macroduct/QPIT in our population, but confidence intervals are wide, although similar for both methods. Larger studies are needed to confirm the sensitivity and optimal cut-off levels for the Nanoduct.[25] We do agree with the proposal of LeGrys[25] to cumulate the results of small studies from over the world to provide solid evidence for the definitive approval of the Nanoduct as accepted sweat test method for the diagnosis of CF.

Conclusion

The success rate of the Nanoduct in our population was 14% higher than that of the Macroduct/QPIT. The Nanoduct significantly discriminated between CF and no CF, and detected the same CF patients as the gold standard methods. The conductivity measured by the Nanoduct correlates well with the chloride concentration of the QPIT/Macroduct. The Nanoduct sweat test system is much easier to use and takes less time. It is feasible and seems reliable as a first diagnostic test after newborn screening.

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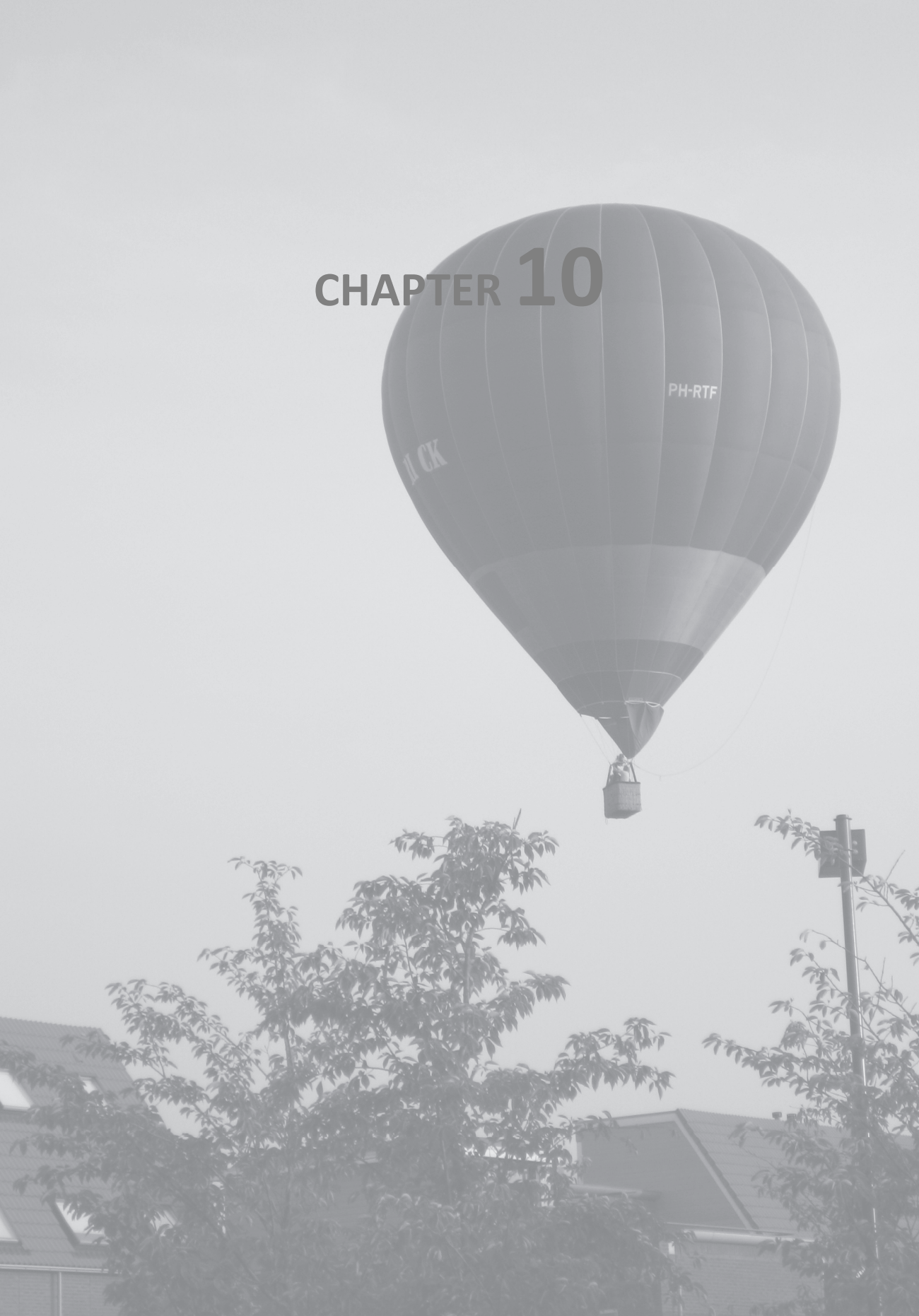
CHAPTER 10



General discussion

General discussion

In the publication of "The principles and practice of mass screening for disease" (1968), Wilson and Jungner formulated, some basic questions that have to be answered before a newborn screening programme can be implemented (Table 10.1).[1] Although defined more than 40 years ago, these criteria are still relevant for newborn screening today. The World Health Organisation (WHO) added another ten criteria in 2008 (Table 10.2).[2]

Table 10.1 Classic screening criteria formulated by the WHO in 1968.[1]

1.	The condition should be an important health problem.
2.	There should be an accepted treatment for patients with recognized disease.
3.	Facilities for diagnosis and treatment should be available.
4.	There should be a recognizable latent or early symptomatic stage.
5.	There should be a suitable test or examination.
6.	The test should be acceptable to the population.
7.	The natural history of the condition should be adequately understood.
8.	There should be an agreed policy on whom to treat as patients.
9.	The cost of case-finding should be economically balanced in relation to possible expenditure on medical care as a whole.
10.	Case-finding should be a continuing process.

Table 10.2 Additional criteria proposed by the WHO in 2008.[2]

11.	The screening programme should respond to a recognized need.
12.	The objectives of screening should be defined at the outset.
13.	There should be a defined target population.
14.	There should be scientific evidence of screening programme effectiveness.
15.	The programme should integrate education, testing, clinical services and programme management.
16.	There should be quality assurance, with mechanisms to minimize potential risks of screening.
17.	The programme should ensure informed choice, confidentiality and respect for autonomy.
18.	The programme should promote equity and access to screening for the entire target population.
19.	Programme evaluation should be planned from the outset.
20.	The overall benefits of screening should outweigh the harm.

In the Netherlands, newborns have been screened for phenylketonuria since 1974, for congenital hypothyroidism since 1981 and for congenital adrenal hyperplasia since 2000. In 2005 the Health Council of the Netherlands advised about expanding our newborn screening programme.[3] The Health Council distinguished three categories of diseases; 1) diseases where screening prevents substantial, irreversible damage, 2) diseases for which screening might prevent damage to a lesser extent, or whereby the benefit is insufficiently proven, and 3) conditions where no health damage is prevented by newborn screening. In 2007, our newborn screening programme was

expanded with 13 metabolic diseases and sickle cell disease.[3] At that time cystic fibrosis (CF) was thought to be a category 2 disease. Treatment of CF leads to substantial health benefits. However, there was some debate about the contribution of newborn screening to the prognosis of the disease (chapter 2).
The Health Council of the Netherlands recommended CF testing to be implemented in our newborn screening programme on the proviso that a reliable method with a high specificity should become available. This recommendation inspired the start of the CHOPIN study (Cystic fibrosis Heel prick amOng a newborn Population In the Netherlands) and in the end to the implementation of CF screening in 2011.[4]

Newborn screening for CF started with a pilot study in part of the Netherlands in 2008 and 2009 (chapter 3). Two novel strategies, immunoreactive trypsinogen (IRT)-pancreatitis-associated protein (PAP) and IRT-DNA-sequencing, were investigated for reliability and test characteristics. After discussing the positive and negative points of both strategies we came up with the idea of a combined strategy, IRT-PAP-DNA-sequencing, which might combine the benefits of both strategies and diminish the negative aspects. This strategy was analysed post hoc, using data of the CHOPIN study.
The criteria for mass screening determined by Wilson and Jungner in 1968 (shown in Table 10.1) were complemented with supplemental criteria made by the WHO in 2008 (shown in Table 10.2).[2] We will take a closer look at those criteria in the context of newborn screening for CF.

1. The condition should be an important health problem

CF is the most common inherited autosomal recessive disease in the Caucasian population. The incidence in the Netherlands is about 1:4750 and the disease causes a considerable morbidity and early mortality in several countries worldwide, which makes it an important health problem.[5] In the past decades, survival improved significantly, mainly because of better treatment options. Whereas in 1968 the median survival of CF was about 8 years, nowadays it is around 45 years.[6,7,8] A more recent review shows that in the US the median predicted survival for infants born between 1980 and 1984 was 37.8 years for males and 31.5 years for females; for males and females born between 1985 and 1994 it was 50.9 and 42.4 years, respectively.[9] The Dutch CF registry shows a median survival of 40 years. The physical complaints, intensive treatment and hospital admissions have a great impact on the quality of life of CF patients, and some patients still do not survive beyond early adulthood.

2. There should be an accepted treatment

CF cannot be cured, but symptoms can be treated. Antibiotics, DNase, hypertonic saline and pancreatic enzymes have proven efficacy in CF. It is known that early treatment leads to a better prognosis, with the best results when diagnosed before the age of two months.[10] In 2010 the newborn screening working group of the European Cystic Fibrosis Society (ECFS) developed guidelines for treatment of infants detected by newborn screening.11 In the Netherlands we have a guideline for diagnosis and treatment of children and adults with CF.12 Following the implementation of newborn screening for CF, the Dutch CF centres developed a national management guideline for infants with CF identified by newborn screening.

3. Facilities for diagnosis and treatment should be available

Every resident in our country is obliged to have a basic health insurance, which leads to accessible care for everybody. Facilities for diagnosis and treatment are well organised in the Netherlands. CF care in the Netherlands is performed by seven CF centres spread across the country, taking care of about 1700 CF patients. All required facilities for diagnosis and treatment of CF are available in these centres, with the exception of lung transplantation which is only possible in three centres.

4. There should be a recognizable latent or early symptomatic stage

Infants with CF are healthy at birth, except when they have a meconium ileus. They mostly develop symptoms within a few months. When an infant becomes symptomatic, irreversible lung damage may already have developed. Newborn screening makes it possible to detect infants before that stage. Sweat of newborns with CF already contains high chloride concentrations, but only symptoms or a positive screening test will lead to performance of a sweat test.

Without screening, in the Netherlands, the median age of diagnosis is about six months. This decreases to four weeks of age with screening (chapter 8). In 13-17% of newborns with CF the diagnosis is made early because of a meconium ileus.[13]

Most infants with CF have early respiratory symptoms: recurrent lower respiratory tract infections, (chronic) cough, dyspnoea, and wheezing. The problem is that many young infants have recurrent (viral) airway infections with respiratory symptoms. This troubles the decision of the general practitioner, when to refer a child for further testing, and of the paediatrician when to do a diagnostic test for CF. In the case of failure to thrive, a doctor may think of CF as a possible cause, but a lot of other causes

should be considered as well. We showed that even when the diagnosis is made early after screening infants already had symptoms of growth failure, however respiratory symptoms were rare in this group (chapter 8).

5. There should be a suitable test or examination.

5.1 Screening strategy

There is a variety of screening protocols worldwide. A European survey was published in 2007, and another overview in 2010.[14,15] Results for sensitivity, specificity and positive predictive value (PPV) of different screening programmes are shown in Table 10.3 and 10.4. The screening programmes are differing substantially; cut-off levels and included mutations vary from programme to programme.

Table 10.3 Results of screening programmes for CF based on phenotype tests.

Author	Year	Programme	Number of screened newborns	Sensitivity (%)	Specificity (%)	PPV (%)
Pederzini et al.[47]	1990	IRT	113,302	96.1	98.4	1.4
Gregg et al.[48]	1993	IRT	220,862	87	99.9	12.5
Larsen et al.[49]	1994	IRT	19,992	92	99.6	9.2
Ryley et al.[50]	1988	IRT-IRT	128,578	90	99.7	8.4
Roberts et al.[51]	1988	IRT-IRT	108,424	70.8	99.9	20
Wesley et al.[52]	1989	IRT-IRT	210,751	92.3	99.99	7.1
Hammond et al.[53]	1991	IRT-IRT	182,965	92.2	99.96	31.9
Wilcken et al.[54]	1995	IRT-IRT	1,015,000	92	99.9	51.7
Scotet et al.[55]	2000	IRT-IRT	343,765	95	99.0	3.3
Narzi et al.[56]	2002	IRT-IRT	51,844	86	99.97	4.8
Sontag et al.[57]	2005	IRT-IRT	1,153,000	94.6	99.8	4.7
Santos et al.[58]	2005	IRT-IRT-conductivity	456,982	100	99.99	7.6
Sarles et al.[21]	2005	IRT-PAP	204,749	100	98	9.4
Stopsack et al.[59]	2009	IRT-PAP	19,924	100	99.87	10.7
Sommerburg et al.[22]	2010	IRT-PAP	73,759	85.7	99.9	12.2
Vernooij-van Langen et al.[24]	***2012***	***IRT-PAP***	***145,499***	***95.0***	***99.897***	***12.3***

Sensitivity, specificity and PPV are displayed with the number of decimals as shown in the study results or were calculated from the study data if possible. IRT=immunoreactive trypsinogen, PAP=pancreatitis-associated protein, DNA=desoxyribonucleic acid, PPV=positive predictive value

Table 10.4 Results of screening programmes for CF based on a combination of phenotype and genotype tests.

Author	Year	Programme (number of included mutations)	Number of screened infants	Sensitivity	Specificity	PPV
Ranieri et al.[60]	1994	IRT-DNA(5)	88,752	100	92.9	31.5
Wilcken et al.[54]	1995	IRT-DNA(1)	189,000	95	99.9	36.6
Gregg et al.[61]	1997	IRT-DNA(10)	104,308	95	99.9	15.2
Castellani et al.[62]	1997	IRT-DNA(3)	95,553	94	99.97	5.9
Rock et al.[63]	2005	IRT-DNA(1)	509,794	94	99	10
Sommerburg et al.[22]	2010	IRT-DNA(4)	73,759	71.4	99.9	17.9
Comeau et al.[64]	2004	IRT-DNA-IRT	323,506	98.2	99.6	8.2
Corbetta et al.[18]	2002	IRT-DNA(31)-IRT	104,609	100	98.6	10.3
Giusti et al.[65]	2007	IRT-DNA(32)-IRT	619,105	98	99.5	3.4
Munck et al.[19]	2008	IRT-DNA(30)-IRT	2,717,905	96.6	99.89	16.7
Ferec et al.[66]	1995	IRT-DNA(5) (-seq)	32,300	100	99.9	19.6
Vernooij-van Langen et al.[24]	***2012***	***IRT-DNA(35)-seq***	***145,499***	***100***	***100***	***64.9***
Castellani et al.[62]	1997	IRT-lactase-DNA(3)	95,553	100	99.95	4.4
Vernooij-van Langen et al.[24]	***2012***	***IRT-PAP-DNA(35)-seq***	***145,499***	***95***	***100***	***87.5***

Sensitivity, specificity and PPV are displayed with the number of decimals as shown in the study results or were calculated from the study data if possible. IRT=immunoreactive trypsinogen, PAP=pancreatitis-associated protein, DNA=desoxyribonucleic acid, PPV=positive predictive value.

All current screening programmes start with an IRT analysis. In 1979, the protein IRT appeared to be increased in newborns with CF, and IRT could be determined from dried blood spots.[16] Sensitivity, specificity and PPV of only IRT were low. To improve the test results, IRT was repeated 4-6 weeks after birth, if the first IRT was positive. This programme resulted in a comparable sensitivity and better specificity, depending on the cut-off level for IRT, and still leaving room for improvement. After discovering the cystic fibrosis transmembrane regulator (CFTR) gene in 1989, it became possible to screen for CF-causing mutations (DNA-screening) leading to higher PPV's. The sensitivity and specificity depend on the number of mutations in the panel. In these programmes it is important to adjust the mutation panel to the ethnicity of the screened population. The ideal screening programme does not exist yet, but the PPV of 87% in our IRT-PAP-DNA-sequencing programme is very good when compared to other programmes.

A characteristic of DNA-based screening is that healthy carriers of a single CFTR-mutation are detected. Another dilemma is that more mutations in the panel will result in better sensitivity but also more referrals and troubled parents. This will be

more pronounced if not only infants with two, but also infants with a single mutation are referred. To avoid this problem, several different screening programmes followed: IRT-IRT-DNA, IRT-DNA-IRT, IRT-DNA-IRT-extended gene analysis (EGA), with sensitivities between 96.5 and 100% and specificities from 98.6 to 99.8%.[17-19]

In 1999, PAP was discovered to be above normal in newborns with CF.[20] An IRT-PAP screening programme was studied in 2005, resulting in a sensitivity of 100% for classical CF (94% including non-classical CF) and a PPV of 9.4%.[21] A similar programme with different cut-off points for IRT and PAP was studied in Germany in 2010, leading to a sensitivity of 85.7% and a PPV 12.2%.[22] The results for IRT-PAP in our study were comparable; a sensitivity of 95%, a specificity of 99.897% and a PPV of 12.3% (chapter 3). A recent study found that PAP is not yet sufficiently elevated in newborns with CF when screened at 48 hours after birth. PAP concentration increases after that time.[23] Therefore, PAP may be usable in newborn screening for CF, in programmes starting from 72 hours after birth. Before that time, PAP concentrations in CF patients do not differ sufficiently from healthy babies. In our study only 19 infants (no CF) were screened before the age of 72 hours showing no lower PAP concentration. We did see an increase in PAP after 168 hours compared to 72-168 hours. In the Netherlands, the heel prick is mostly performed between 72 and 168 hours after birth. Therefore, including PAP in the screening protocol in the Netherlands is feasible.

Our study on PAP also revealed that sex, gestational age and birth weight led to small differences in PAP concentration, but these differences did not lead to recommendations to adjust cut-off levels for certain groups (chapter 4). However, PAP concentrations were clearly higher in low-birth-weight infants (<2000 gram), and after a blood transfusion. We recommend to repeat the heel prick after three months, when an infant received a blood transfusion before the heel prick. This is already recommended for other diseases in our newborn screening programme.

The other strategy in the CHOPIN study, IRT-DNA-sequencing, differed from most global DNA based programmes. Only infants with two mutations were referred to a CF centre for further diagnosis. Infants with a single mutation were considered healthy carriers. The sensitivity and specificity were 100% and the PPV was 64.9% (chapter 3). The PPV is higher than in most screening programmes for other diseases.

To combine the advantages and diminish the disadvantages of both strategies, we performed a post-hoc analysis for an IRT-PAP-DNA-sequencing programme, four steps before parents were informed about a positive screening test. This has led to a sensitivity comparable to the IRT-PAP strategy and comparable to other screening programmes. On the other hand the number of detected carriers and equivocal diagnosis (and thus false-positives) decreased. Again, only infants with two mutations were referred for further diagnosis. In this combined strategy one infant with CF was

missed because of a low PAP concentration.[24] The sensitivity of this strategy was 95%, similar to IRT-PAP; the specificity was 100% and the PPV 87%.
The IRT-PAP-DNA-sequencing strategy was implemented in the Netherlands in 2011. A failsafe procedure was added for infants with a very high IRT and no mutations, because in the Netherlands we have a Turkish and North African population with rare mutations that are not within our panel and would possibly be missed.[25]

5.2 Diagnostic test

The gold standard test to confirm the diagnosis of CF is the sweat test. Sweat chloride concentrations higher than 60 mmol/l in combination with a clinical picture are pathognomonic for CF.[26] Chloride concentrations between 30 and 60 mmol/l are not conclusive. CF can also be confirmed by DNA mutation analysis. Over 1900 mutations are known to cause CF or CFTR related disease (equivocal CF diagnosis, non-classic CF).[27]

Performing a sweat test in infants younger than two months of age is a challenge. Young infants sweat less, and collection of a sufficient amount of sweat is difficult. The gold standard methods Quantitative Pilocarpin Ionthophoresis Test (QPIT) and Macroduct show success rates between 73.8 and 99.3% for all ages, and less in young infants (Table 10.5). This means that in a considerable number of referred infants the sweat test needs to be repeated, which means a longer stressful period for the parents. The Nanoduct sweat test system needs less sweat and was especially developed for young infants. The Nanoduct is much easier to perform and takes less time. The success rate of the Nanoduct in our study was 95%, which was significantly better than the reference tests, QPIT and Macroduct (chapter 9). Although the QPIT performed well in most studies, including ours, this method is much more laborious. Performing a QPIT needs more trained and experienced personnel, and takes more time to get a test result.

The Nanoduct proved to be reliable in detection or exclusion of CF in young children (chapter 9). The internationally recommended cut-off points for conductivity are 60 mmol/l and 90 mmol/l, which correspond to the ideal borders in our study.[28] We did not investigate the cost-effectiveness of the Nanoduct but expect this to be good. We think the Nanoduct will be useful in confirming the diagnosis after a positive screening test. Advantages are the direct result at the first appointment at the CF centre. With two mutations and a positive Nanoduct test, the parents can be informed and counselled about the diagnosis right away. Infants with an equivocal Nanoduct result should be re- tested, just like after an equivocal gold standard test result.

Table 10.5 Success rates of QPIT, Macroduct and Nanoduct in different populations.

Author	Year	No. of subjects	Age	QPIT	Macroduct	Nanoduct
Hammond et al.[67]	1994	1090	3 days to 78 years	99.3%	93.9%	
Farrell et al.[68]	1996	725 (481 after NBS)	all ages	99.3%		
Massie et al.[69]	2000		6-8 weeks		96.5%	
Heeley et al.[70]	2000	211	6 month to 15 years		98.6%	
Mastella et al.[71]	2000	318	24 days to 46 years	96.4%	90.9%	
Taccetti et al.[72]	2004	1003	6-8 weeks	92.9%		
Parad et al.[73]	2005	1214	2 weeks 3-8 weeks	83% 89-97%		
Eng et al.[74]	2005	103	<6 weeks >6 weeks	73.8% 98.4%		
Barben et al.[75]	2005	111	3 weeks To 60 years		84.7%	97.3%
Losty et al.[76]	2006	100	all ages			97%
Desax et al.[77]	2007	1041	<1 months to >16 years		81.6%	90.9%
Laguna et al.[78]	2012	568	after NBS	84.6%	97.9%	
Vernooij-van Langen et al.[24]	***2013***	***108***	***<2 months after NBS***		***79%***	***94%***
Barben et al.[79]	2013	162	after NBS		74%	86%

NBS=newborn screening

6. The test should be acceptable to the population

CF was added to the routine newborn screening programme, and no extra blood was needed to perform all the four steps in the screening strategy. So no extra efforts are necessary for parents, children or health care workers. In this programme parents are only aroused when there is a very high suspicion of their child having CF: High IRT and PAP and the presence of two mutations. There are no real false-positives (healthy infants with a positive screening result), although some infants with an equivocal CF diagnosis are discovered. Some of those infants might stay asymptomatic during their whole life, but others might develop symptoms later in life, which will be recognized earlier. A possible disadvantage of screening including DNA mutation analysis is detection of healthy carriers, although one may consider this as a benefit. We asked pregnant couples for their opinion on this subject, in focus group discussions. Most of them (87%) wanted to be informed, but they all agreed parents should have the choice not to know (chapter 7). In our screening programme parents were asked if they wanted to be informed about the carrier status of their child when screening would detect this. This was noted on the heel prick card by the screener (person that

performs the heel prick). In our screening programme a very small number of carriers was detected: about 8 per 150,000 screened infants. We also asked parents of infants with a false positive screening test about their opinion on newborn screening for CF, and almost all were positive and would participate again in a next pregnancy (chapter 6).

In general, Dutch parents are very positive about newborn screening, which also holds for newborn screening for CF. The screening is something you just do for your baby. Parents want to know everything that can be detected before the child will become symptomatic, even when the disease is not treatable.[29] There is a high acceptance of newborn screening in the Netherlands.

Well-informed parents showed less stress and anxiety (chapter 6). Education of the parents is very important. They should receive information about newborn screening, CF, detection of carriers and disclosure of the carrier results, and about the follow-up after a positive screening test. Parents need to understand the subject to make an informed choice whether or not to participate in newborn screening and to be informed about the carrier state of their child. We developed a leaflet, which was available in nine languages. We also developed a website with additional information and links to other websites, that was used by 48% of parents of a child with a (false-)positive screening result and 7% in the screen negative group (chapter 6).

7. The natural history of the condition, including development from latent to declared disease, should be adequately understood

The natural course of CF is known.[30] The genetic defect was discovered in 1989. There is a variable clinical expression of the disease, even within infants with the same mutations.[31] The role of modifier genes, gene-gene interactions, other channels than the chloride channel and environmental factors is not completely understood and still subject of on-going study. Newborn screening provides an opportunity for better understanding the course of the disease and the influence of early treatment.

8. There should be an agreed policy on whom to treat as patients

Infants are referred to a hospital for a sweat test because of clinical features of CF, a positive family history and/or a positive newborn screening test.[32] CF is confirmed in infants with two or more phenotypic characteristics or two known CF-causing mutations and a sweat chloride concentration of 60 mmol/l or higher.[26] Infants with an

equivocal diagnosis after newborn screening are infants with one or two mutations of unknown clinical significance and a normal (chloride < 30mmol/l) or equivocal (chloride 30-60 mmol/l) sweat test.[33] Follow-up of infants with an equivocal diagnosis is needed to assess potential disease.

9. The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole

Screening for CF proved to be cost-effective when compared to diagnosis based on clinical symptoms. This was previously studied for four strategies (IRT-IRT, IRT-DNA, IRT-DNA-IRT and IRT-DNA-denaturing gradient gel electrophoreses (DGGE)). IRT-IRT had the most favourable cost-effectiveness ratio of €24,800 per life-year-gained. IRT-DNA-DGGE resulted in more health effect at lower costs than IRT-DNA-IRT.[34] In our study, we showed that the cost-effectiveness ratio for IRT-PAP, IRT-DNA-sequencing and IRT-PAP-DNA-sequencing varied from €23,600 to 29,200 per life-year-gained. IRT-PAP had the most favourable cost-effectiveness ratio compared to no screening. Additional life-years could be gained by the IRT-DNA-sequencing strategy but against higher costs. We concluded that newborn screening for CF is economically justified. All screening strategies may result in cost-savings when early diagnosis and treatment lead to a 5% reduction in lifetime costs of treatment (chapter 5).

10. Case-finding should be a continuing process

Our newborn screening programme in the Netherlands started in 1974 with PKU and is an on-going programme, expanded over the years to a total of, at present, 18 diseases. CF was included since 2011. Participation in newborn screening is 99.7% in our country, so nearly all newborns are tested. Parents are educated about the usefulness of newborn screening and about the diseases their baby will be screened for. Therefore, case-finding is an on-going process in the Netherlands.

11. The screening programme should respond to a recognized need

To get more information about the opinion of parents about newborn screening, 396 mothers were asked for their opinion in 2008.[29] Almost 50% of the questionnaires were returned, showing that parents were very positive about expanding of our

newborn screening programme. Rare diseases or high numbers of false-positive results were of subordinate importance to them. Similar results were seen for untreatable diseases. In our study most parents wanted to be informed when the test revealed their child as healthy carrier (chapter 7). Many parents in our study had the opinion that newborn screening belongs to everything that is normally done when a baby is born. They noted this in the note box in their questionnaires of the study on the effects of false-positive tests (chapter 6).
During our study we evaluated the effect of our education programme with a questionnaire for parents. Parents were very positive about implementation of CF screening. It looks like parents just want to know as much as they can before their child will get symptoms. Paediatricians worldwide are in favour of screening, because early treatment leads to health benefits and an early clinical diagnosis can be difficult to make. Professional associations as the Dutch Paediatric and Cystic Fibrosis Society (NVK and NCFS), ECFS and Cystic Fibrosis Foundation (CFF-USA) are all in favour of newborn screening. This means that newborn screening for CF fulfils this need.

12. The objectives of screening should be defined at the outset

The objective of newborn screening is to detect children with CF directly after birth.[35] In the Netherlands, almost all infants are screened between day 4 and 7 after birth. Our screening programme is offered to infants of maximum 6 months of age, to include infants arriving in the country as a result of adoption, but their number is small.

13. There should be a defined target population.

The aim of newborn screening for CF was defined as detection of infants with classical CF, infants that will benefit most when diagnosed before the age of two months and before they develop symptoms or irreversible lung-damage.

14. There should be scientific evidence of screening programme effectiveness

The effectiveness of newborn screening has been proven in many studies over the years. In the CHOPIN study we showed the effectiveness of three novel screening strategies. All three strategies, IRT-PAP, IRT-DNA-sequencing and IRT-PAP-DNA-sequencing, achieved comparable results to existing programmes for sensitivity.

Specificity and PPV were better for IRT-DNA-sequencing and IRT-PAP-DNA-sequencing strategies (chapter 3).

15. The programme should integrate education, testing, clinical services and programme management

In the Netherlands, the newborn screening programme is coordinated by the Centre for Population Screening (CVB), part of the National Institute for Public Health and the Environment (RIVM). We have a national education programme for parents and also for health care employees participating in newborn screening. Leaflets about newborn screening are available in Dutch and nine other languages and there is information on the website (www.rivm.nl/hielprik). Parents receive the written information at three occasions, and are informed verbally by their midwife or gynaecologist. At the start of our study we educated all screeners (people that perform the heel prick) verbally, on a voluntary base. All midwifes, gynaecologists, general practitioners and paediatricians in the study region received written information.
The screening programme was studied thoroughly before nationwide implementation. CF centres were prepared for taking care of the referred infants. During the study the CF programme was managed by a research nurse and researcher-paediatrician with the help of the people managing the routine programme. After implementation this was taken over by the Centre for Population Screening (CVB). As screening for CF was added to a routine newborn screening programme, all the experience and resources were available.

16. There should be quality assurance, with mechanisms to minimize the potential risk of screening

The IRT-PAP-DNA-sequencing programme was implemented in the Netherlands because of the high specificity and PPV and the low number of detection of equivocal diagnosis and carriers (chapter 3). The sensitivity was acceptable. From July 2007 until January 2012 Dutch paediatricians reported all children with a new CF diagnosis to the Dutch Paediatric Surveillance Unit (DPSU), and since January 2012 only infants missed by screening are reported. The newborn screening programme is monitored by a permanent steering committee in which the various professional disciplines and institutes involved in the programme are represented. The steering committee is responsible for the annual process and effect evaluation of the programme. The screening programme is evaluated every year and adjusted to new insights when needed.

17. The programme should ensure informed choice, confidentiality and respect for autonomy

Participation in our newborn screening programme is not obligatory, parents may "opt out" when they do not want their child to be screened. Very few parents do so, about 0.3%. Registration of the personal information is protected by legislation and controlled by the college of protection of personal data (CBP). Personal data are only provided for identification of screen-positive infants who need referral for further diagnostic testing. All other screening results are stored without any direct accessible link to the personal data.

In focus group discussions we asked parents about their opinion on disclosure of carrier results, a consequence of DNA-based screening. Most parents wanted to be informed about the results, but the general opinion was that parents should have the right not to know. At this point parents need to say if they want to be informed if their child turned out to be a carrier of CF. Parents can indicate on the heel prick card whether they want to be informed about the carrier status of their child or not (chapter 7).

18. The programme should promote equity and access to screening for the entire target

All newborns in the Netherlands have access to newborn screening. It is a nationwide programme and parents do not have to pay for it.

19. Programme evaluation should be planned from the outset

See above under point 16.

20. The overall benefits of screening should outweigh the harm

There has been a long debate about the benefits of newborn screening for CF, but nowadays we may conclude that the benefits outweigh the harm. We found that newborns, when detected at the age of one month, have significantly less pulmonary symptoms, steathorroea, ear-nose-throat problems and hospital admissions at diagnosis than children with CF who are diagnosed on clinical symptoms (chapter 8). Early diagnosis by newborn screening prevents malnutrition and therefore promotes better growth and development.[36,37] Better growth will also lead to better lung

function and a better prognosis.[38] Vitamin deficiency can be prevented. Lower airway tract infections can be treated sooner and more aggressively, leading to better preservation of lung function.[39] Newborn screening will also lead to less hospital admissions and less invasive treatments.
The survival improves when diagnosed before the age of two months.[8,10] Diagnosis by newborn screening may also lead to cascade family screening and possibly influences future family planning. In focus groups almost all participating Dutch parents stated that they would ask for genetic counselling in case their child would be discovered as a carrier (chapter 7).

One of the disadvantages of screening is the stressful period between the positive screening test and the final diagnosis. In our screening programme the diagnosis is almost certain when the screening is positive, as only infants with two mutations are referred for further diagnosis. Parents are informed about the positive screening for CF the day before their appointment at the CF centre. The paediatric pulmonologist knows the result of the mutation analysis and informs the parents about the disease. The result of the sweat test will be known the same day, to keep this difficult period as short as possible.
Another disadvantage is false-positive test results. Although parents showed highly negative feelings of stress and concern after the positive test and those feelings have disappeared after six months, false-positive results should be kept at a minimum (chapter 6).
IRT-PAP screening leads to false-positive results. Infants may show high IRT and PAP levels originating from other causes, such as neonatal infection or hypoxia. Most of them will have a normal sweat test. In our IRT-PAP-DNA-sequencing strategy there are no real false-positive results, because only infants with two mutations were referred. Even when infants with a single mutation would be referred, they are healthy but carrier of the disease which might have implications for their family and future. Detection of healthy carriers might be seen as a disadvantage, arousing parents of a healthy child. Many parents and health care providers see carrier detection as a benefit, because parents can think about further family planning and other family members can be tested as well.

A problem of our IRT-PAP-DNA-sequencing programme is detection of equivocal diagnosis, infants with one or two mutations with unclear clinical consequences. In France, R117H is the second most prevalent CFTR mutation, only found after screening and not because of clinical symptoms.[40] We detected thirteen infants with R117H-7T that had normal sweat tests and no symptoms. Most people with a second R117H-7T mutation will never have any symptoms, or mild to moderate pulmonary problems

later in life. It is questionable if these infants benefit from newborn screening or are harmed by it. For this reason, there are countries that skipped the R117H from their screening panel. It is difficult to counsel parents, not being able to predict if their child will get symptoms and when.[41,42] There is discussion about categorising those infants as having "mild" CF and seeing them in follow-up, as they might stay asymptomatic for years.[40,43] There are case-reports about R117H-7T infants with pulmonary symptoms.[44,45] R117H is associated with male infertility, but knowledge of this from birth is not very beneficial. In our programme we only detect a small number of infants with an equivocal diagnosis. We decided to see all infants in follow-up so we could learn more about the natural course of those mutations.

False-negative screening results are a real problem, but a 100% sensitive screening test will lead to high false-positive rates and costs. False-negative results might lead to a diagnostic delay when the child develops symptoms. Rare mutations in a certain population might be missed. That is why we incorporated a failsafe procedure to detect infants with two rare mutations. In 2013 three infants with CF were missed because of large deletions not detected by sequencing. Since then, also infants with a single mutation are referred for a sweat test. After three years we will decide whether this adjustment was really needed.

According to the ethical principles in medicine and the oath of Hippocrates, there are four important principles: 1) doing good, 2) doing no harm, 3) the right to choose, and 4) being fair and equitable. Those unifying ethical principles form the basis of a report on protection of human subjects of research, the Belmont report.[46] Newborn screening for CF in the Netherlands fulfils these criteria. It is accessible for everyone, as is the care for patients. Parents and health care workers are educated. Parents have the right to participate in newborn screening or opt out. They also can choose whether they want to know carrier results. We have a sensitive and highly specific screening programme. Infant data are processed with care. We conclude that the benefits of NBS for CF outweigh the harm of newborn screening for CF, we are doing good and little harm.

Directions for future research

The treatment of infants with CF is based on a consensus statement that was written in 2010.[11] This has to be evaluated and the Dutch CF centres developed a protocol about follow-up requirements and research for this cohort of screened and mostly asymptomatic group. We will be able to compare screened infants with CF to a clinically diagnosed group of patients over the years. The role of early therapeutic intervention on quality of life and survival will be very interesting. The role of early CT scanning, lung function tests and bronchoscopy at a young age is unclear. It is also unknown if early treatment with hypertonic saline, DNase or antibiotics will lead to a better outcome in this group.

Future investigations will lead to increased knowledge about CFTR function and its role in different organ systems, modifier genes, environmental influences and gene-environment interactions in CF on the course of the disease. New treatment opportunities will become available, which will be especially important for this group of patients.

Intestinal current measurements (ICM) in order to determine CFTR rest function in infants with an equivocal diagnosis may lead to prediction of development of symptoms in those infants. ICM may help to develop a more differentiated follow-up protocol for this group.

Airway inflammation has a central role in the pathogenesis of CF. Measurement of inflammation markers in sputum, exhaled breath, or blood, and expression of inflammation genes may play a role in optimising the management of the disease, which is the topic of current research.

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Chapter 11

Summary / Samenvatting

Summary

Cystic fibrosis (CF) is one of the most common inherited diseases in the Netherlands and other western countries, with around 1 in 4750 newborns affected in the Netherlands. Pathogenic mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, encoding for a chloride transporter protein in the membrane of epithelial cells, cause CF. CF is a multi-organ disease. The first symptoms of CF in infants are often feeding difficulties, failure to thrive and recurrent airway infections. Those complaints often occur in young infants, which may lead to a delay of the CF diagnosis. In 13-17% of the cases the diagnosis is made early after birth because of a meconium ileus. Newborn screening facilitates early detection.

In **chapter 2** we review the benefits and disadvantages of newborn screening for CF (NBSCF). An early diagnosis leads to a better growth and nutritional status by treatment of the pancreas insufficiency with digestive enzymes and vitamin supplements. Observational evidence shows that an early diagnosis leads to a better preservation of lung function into adulthood. Early treatment of infections may prevent lung damage and leads to less and shorter hospital admissions and less invasive therapies. NBSCF may also improve survival. However, NBSCF also has drawbacks. The occurrence of false-positive and false-negative results are a disadvantage of screening in general. A disadvantage specifically for CF is detection of healthy carriers and infants with an equivocal diagnosis. The time between the positive screening test result and confirmation or exclusion of the diagnosis is very stressful for the parents and should be kept as short as possible.

NBSCF is included in many routine newborn screening programs in other countries, but current strategies have considerable drawbacks such as false-positive tests, equivocal diagnosis and detection of carriers. In **chapter 3** we show the results of the CHOPIN-study (Cystic fibrosis Heel prick amOng a newborn Population In the Netherlands), which investigated three novel screening strategies. In the first strategy, concentrations of immunoreactive trypsinogen (IRT) and pancreatitis-associated protein (PAP) were measured. In the second strategy, samples with IRT ≥60 µg/l were analysed for 35 CFTR mutations, followed by sequencing of the whole CFTR gene when a single mutation was detected. Tests were only positive when two CFTR mutations were identified. The IRT-PAP approach showed a sensitivity of 95.0% with a specificity of 99.897%, and a positive predictive value (PPV) of 12.3%. Test properties for the IRT-DNA-sequencing strategy were respectively 100%, 100%, and 64.9%. Combining both strategies, the third strategy (IRT-PAP-DNA-sequencing), led to a sensitivity of 95.0%, a specificity of 100%, and a PPV of 87.5%. In conclusion, all strategies performed well.

Although there was no statistical significant difference in test performance, the IRT-DNA-sequencing strategy detected one more infant, that was missed by IRT-PAP and IRT-PAP-DNA-sequencing. IRT-PAP may be the optimal choice if the use of DNA-technology must be avoided. If identification of carriers and equivocal diagnosis is considered an important disadvantage, IRT-PAP-DNA-sequencing may be the best choice.

PAP is currently discussed as a marker in NBSCF. In **chapter 4** we show that that sex, birth weight, and gestational age were accompanied by small differences in PAP concentrations without consequences for the screening algorithm. However, blood transfusion as well as performance of the heel prick after 168 hours (7 days) led to clinically significant higher PAP levels and to a higher risk on a false-positive screening test result.

In **chapter 5** we compare costs and effects of three novel screening strategies (IRT-PAP, IRT-DNA- sequencing and IRT-PAP-DNA-sequencing) with a situation without screening and with IRT-DNA, which is worldwide the most used screening strategy. Compared to a situation without screening, screening strategies had cost-effectiveness ratios (CER) varying from €23,600 to €29,200 per life-year gained. The IRT-PAP strategy had the most favourable CER. Additional life-years can be gained by the IRT-DNA strategy but against higher costs. In a situation of 5% reduction in treatment costs, screening even leads to financial savings.

Chapter 6 describes that parental anxiety induced by a false-positive screening result disappeared after six months and that negative parental feelings could be reduced by education. A positive screening test result induces parental anxiety but false positive test results in NBSCF do not cause long-term anxiety. Well-informed parents show lower stress and anxiety levels.

Most NBSCF programs also identify healthy carriers. In **chapter 7** we explored the opinion of pregnant couples about the disclosure of the carrier status of children when revealed after newborn screening. Most parents want to be informed when newborn screening shows their newborn being a CF-carrier. Their main reason was the implication of this knowledge for further family planning. Other family members can be informed and children within the family can be tested. Parents stated they have the right to know, but others also expressed that the choice of not being informed should be offered as well.

In **chapter 8** we showed that infants detected after NBSCF are in a significantly better condition at diagnosis than children with a clinical diagnosis (CD), even when the gain in time at diagnosis is only 5-6 months. Growth retardation was already present in 30% of the patients diagnosed by NBSCF, but respiratory disease in only 4 %. Compared with NBSCF, significantly more patients with a clinical diagnosis showed failure to thrive, respiratory symptoms, and hospitalizations. In the CD group, 62% of the patients showed abnormal signs at physical examination compared to 4% in the NBS group. NBSCF leads to an early diagnosis before respiratory symptoms have developed.

After a positive screening result for CF, a sweat test is performed to confirm the diagnosis. The mean estimated success rate of the generally acknowledged methods (Macroduct/QPIT) in newborns is about 87%. The Nanoduct sweat test system is easier to perform and less sweat is needed. In **chapter 9,** a comparison between the effectiveness of the Nanoduct and the gold standard methods (Macroduct/QPIT) is described. We found a success rate of 93% for the Nanoduct which was significantly better than 79% for the Macroduct/QPIT. The Nanoduct detected the same CF patients as the Macroduct/QPIT, one CF patient had an equivocal result for both tests, and no patient was missed. The ideal cut-off points for conductivity were comparable to former studies, 91 mmol/l and 66 mmol/l. The Nanoduct is a reliable method to confirm or exclude the diagnosis CF after newborn screening.

This thesis contributes to the knowledge about newborn screening for cystic fibrosis. We investigated three new screening strategies for test qualities and costs, the effect of newborn screening on the clinical condition at diagnosis, the feasibility of a novel sweat test, the impact of false-positive test results on parents, and the opinion of parents about disclosure of carriers after NBSCF. In **chapter 10** the conclusions from these studies are discussed against the ethical background of the principles of mass screening for disease formulated by Wilson and Jungner, and the WHO. Newborn screening for CF in the Netherlands fulfils these criteria. It is accessible for everyone, as is the care for patients. Parents and health care workers are educated. Parents have the right to participate in newborn screening or opt out. They also can choose whether they want to know carrier results. We have a sensitive and highly specific screening programme. Infant data are processed with care. We conclude that the benefits of NBS for CF outweigh the harm of newborn screening for CF, we are doing good and little harm.

Samenvatting

Cystic Fibrosis (CF), ook wel “taaislijmziekte” genoemd, is één van de meest voorkomende erfelijke aandoeningen in de Westerse bevolking en komt in Nederland voor bij ongeveer één op de 4750 pasgeborenen per jaar. CF wordt veroorzaakt door twee mutaties (veranderingen in het erfelijk materiaal (DNA)) in het cystic fibrosis transmembraan regulator (CFTR)-gen.
Het CFTR-gen zorgt voor de productie van een chloorkanaal in oppervlaktecellen van de huid en de slijmvliezen. Dit chloorkanaal zorgt voor transport van water en zout door de cel. Bij slecht functioneren van het chloorkanaal ontstaat abnormaal dik en taai slijm in verschillende organen waaronder de luchtwegen en het maagdarmkanaal.

Er ontstaan slijmophopingen in de kleine luchtwegen leidend tot chronische infecties en onherstelbare longschade, met als resultaat een kortere levensverwachting. In de alvleesklier leidt het taaie slijm tot verstopping van de afvoerkanalen. Daardoor kunnen de verteringsenzymen niet in de darm komen en worden voedingsstoffen onvoldoende opgenomen met als gevolg vette diarree en ondervoeding. Het zweet van personen met CF bevat meer zout dan normaal. Door middel van een zweettest kan dit worden gemeten. Hiermee kan de diagnose CF worden vastgesteld.

De eerste verschijnselen van CF zijn voedingsproblemen, slecht groeien en herhaalde luchtweginfecties. Dit zijn klachten die veel voorkomen bij jonge kinderen waardoor CF niet altijd vroeg wordt herkend. Door het hielprikonderzoek van pasgeborenen uit te breiden met CF, kan de diagnose op jonge leeftijd worden vastgesteld.

Voor- en nadelen van screening van pasgeborenen op CF

Voordelen van screening zijn een eerdere diagnose en het vroeg kunnen starten van de behandeling. Door toediening van verteringsenzymen en een energierijk dieet kan ondervoeding worden voorkomen. Een goede voedingstoestand leidt tot een betere conditie van de longen. Door snellere en agressievere behandeling van infecties wordt schade aan de longen beperkt of uitgesteld; de longen functioneren langer goed. Screening heeft ook als gevolg dat minder ziekenhuisopnames nodig zijn en leidt zelfs tot een betere overleving op de kinderleeftijd. Verder blijft kinderen en ouders een lang traject met onderzoeken, ziekenhuisopnames en veel onzekerheid bespaard, zoals vaak het geval is als de diagnose wordt gesteld op basis van luchtwegklachten of slecht groeien.
Aan de andere kant is een nadeel van screening dat ook kinderen worden gevonden met een afwijkende hielprik die later geen CF blijken te hebben (een fout-positief resultaat). Dit maakt ouders ongerust terwijl dat achteraf niet nodig blijkt te zijn. Ook

worden dragers gevonden van CF, kinderen met één mutatie in het CFTR-gen en één normaal gen, dragers van CF zijn gezond. Door screening worden ook kinderen met een milde variant van CF ontdekt. Deze kinderen krijgen waarschijnlijk geen of alleen milde luchtwegklachten op volwassen leeftijd. Zij hebben dus geen baat bij een vroege diagnose. Uiteindelijk is in 2005 door de Gezondheidsraad besloten dat de voordelen van screening op CF in principe opwegen tegen de nadelen en dat ook in Nederland pasgeborenen op CF moeten worden onderzocht. De Gezondheidsraad vond wel dat er meer onderzoek nodig was om de nadelen zo beperkt mogelijk te houden en de voordelen te optimaliseren. Dit was de aanleiding voor de CHOPIN studie (Cystic fibrosis Hielprik Onderzoek bij Pasgeborenen In Nederland) welke een belangrijk onderdeel is van dit proefschrift.

Nieuwe screeningsstrategieën

In de CHOPIN studie worden drie nieuwe screeningsstrategieën met elkaar vergeleken:

1) Twee biochemische testen: Bepaling van het immunoreactief trypsinogen (IRT), een eiwit dat wordt geproduceerd door een beschadigde alvleesklier, zoals het geval is bij CF. Gevolgd door bepaling van het pancreatitis-associated protein (PAP), een eiwit dat vrijkomt uit een alvleesklier onder stress.
2) Een IRT, gevolgd door bepaling van 35 veel voorkomende mutaties in het CFTR-gen. Bij detectie van slechts één mutatie, volgt verder onderzoek naar een tweede mutatie in het hele CFTR-gen door middel van sequencing.
3) Een combinatie van beiden; IRT, gevolgd door PAP. Als beide verhoogd zijn volgt de mutatie analyse en bij detectie van slechts één mutatie ook sequencing.

Dit proefschrift laat zien dat de IRT-DNA-sequencing (2) strategie de meeste kinderen met CF opspoort, maar deze strategie vindt ook relatief veel gezonde dragers en kinderen met een milde variant van CF. De combinatie strategie, IRT-PAP-DNA-sequencing (3), vindt 95% van de kinderen met CF, maar minder gezonde dragers en minder kinderen met een milde variant van CF. Daarmee combineert deze strategie de voor- en nadelen van screening op CF het beste.
De resultaten van dit deel van de CHOPIN-studie was aanleiding voor een volgend advies van de Gezondheidsraad. De Gezondheidsraad adviseerde in 2010 de IRT-PAP-DNA-sequencing strategie in Nederland in te voeren. Hierop besloot de Minister van Volksgezondheid om de screening op CF per 1 mei 2011 in te voeren als onderdeel van de neonatale hielprikscreening.

Factoren die van invloed zijn op het PAP eiwit

PAP is verhoogd bij pasgeborenen met CF en minder bij pasgeborenen met een milde variant van CF. PAP wordt nauwelijks beïnvloed door geslacht, zwangerschapsduur en geboortegewicht. Echter, afname van de hielprik na 168 uur (dag 7) na de geboorte (in Nederland wordt de hielprik afgenomen tussen dag 3 en 7) en afname na een bloedtransfusie leiden tot verhoogde waarden en daarmee tot meer foutief afwijkende test uitslagen.

Kosten-effectiviteit van screening op CF bij pasgeborenen

Invoering van CF-screening is economisch verantwoord en kan leiden tot een kosten besparing, uitgaande van lagere behandelkosten en minder aanvraag van zweettesten buiten de screening om. De IRT-PAP strategie is het meest kosten-effectief ten opzichte van IRT-DNA(-sequencing) en IRT-PAP-DNA-sequencing.

Impact van een fout-positieve uitslag op ouders en de invloed van kennis

Een fout-positieve uitslag is de situatie dat een kind een afwijkende hielprikuitslag heeft en bij verder onderzoek de ziekte CF niet blijkt te hebben. Fout-positieve uitslagen leiden tot sterk negatieve gevoelens bij ouders en veel onzekerheid, maar zes maanden later zijn deze negatieve gevoelens vrijwel geheel verdwenen. Het is belangrijk ouders goed te informeren over het hielprikonderzoek, de ziekte CF, en de mogelijkheid en betekenis van een fout-positieve uitslag. Goed geïnformeerde ouders laten minder gevoelens van angst en depressie zien.

Ouders wel of niet informeren over dragerschap?

Bij screeningsstrategieën die naar het erfelijk materiaal (het DNA) kijken worden enkele, maar lang niet alle, dragers van CF gevonden. Dragers zijn mensen met slechts één mutatie in het CFTR-gen die gezond zijn. Over de vraag of ouders hierover geïnformeerd moeten worden, voerden we discussies met aanstaande ouders. De meeste ouders (83%) willen worden geïnformeerd over dragerschap bij hun kind, ook al zijn dragers gezond. Dragerschap kan consequenties hebben voor een eventuele volgende zwangerschap. Ouders kunnen dan worden onderzocht op het toekomstig risico op het krijgen van een kind met CF. Ook kunnen andere familieleden worden onderzocht op dragerschap voor CF. Ouders vinden wel dat ze het recht moeten hebben om niet te worden geïnformeerd als zij dat niet willen. Dit moet vooraf aan ouders worden gevraagd.

Verschil in lichamelijke conditie bij diagnose na screening of op basis van klachten

Kinderen met CF opgespoord door screening (op de leeftijd van 3 weken) zijn in een betere lichamelijke conditie bij diagnose dan kinderen gevonden op basis van lichamelijke klachten zelfs als het gemiddelde verschil in leeftijd maar 5-6 maanden bedraagt. Bij een CF diagnose na de hielprikscreening is al enige groeiachterstand zichtbaar, maar de diagnose wordt gesteld voordat luchtwegklachten ontstaan.

De Nanoduct, een nieuwe zweettest methode

Na een afwijkende hielprik wordt de diagnose CF bevestigd door middel van een zweettest. Deze test is bij zeer jonge kinderen (3-4 weken) lastig uit te voeren vanwege een lage zweetproductie. De Nanoduct is een innovatieve zweettest methode die eenvoudiger is uit te voeren en minder zweet nodig heeft dan de internationaal geaccepteerde zweettest methoden. Het succespercentage van de Nanoduct is 14% hoger dan van de standaard zweettesten, terwijl een betrouwbaar onderscheid tussen CF en geen CF met de Nanoduct goed mogelijk is. De Nanoduct lijkt daarmee bruikbaar als vervolgtest na neonatale screening op CF.

De studies in dit proefschrift leveren een bijdrage aan de kennis over screening op CF bij pasgeborenen. Onderzocht werden drie nieuwe screeningstrategieën, de kosteneffectiviteit, het effect op de lichamelijke conditie bij diagnose, details over de biochemische test PAP, de Nanoduct zweettest en de impact van de resultaten van de screening op ouders en kinderen.
De hielprikscreening op CF in Nederland voldoet aan de aan de criteria voor screening zoals geformuleerd door Wilson en Jungner en de WHO.

Curriculum Vitae

Curriculum Vitae

Anna Margaretha Maria van Langen, Annette, werd geboren op 9 augustus 1975 te Heerhugowaard. Zij behaalde in 1993 haar VWO diploma aan het Han Fortmann college te Heerhugowaard. In datzelfde jaar begon zij met de studie Geneeskunde aan de Vrije Universiteit in Amsterdam, waar zij in 2000 haar artsexamen behaalde. Gedurende haar opleiding deed ze wetenschappelijk onderzoek naar kandidaat genen voor type 2 diabetes aan de University of Newcastle upon Tyne in Engeland. In 2001 begon ze als AGNIO (assistent geneeskunde niet in opleiding) in het Atrium Medisch Centrum te Heerlen. Ruim een jaar later startte ze haar opleiding kindergeneeskunde in het Academisch Ziekenhuis Maastricht (tegenwoordig MUMC). Gedurende twee jaar van haar opleiding was Annette werkzaam in het Viecuri Medisch Centrum te Venlo. Zij rondde haar opleiding tot kinderarts af in 2006 waarna zij begon als waarnemend kinderarts in het Lange Land ziekenhuis te Zoetermeer en vervolgens in het Medisch Centrum Alkmaar. Van 2007 tot 2010 deed zij onderzoek bij het Atrium Medisch Centrum onder begeleiding van Jeannette Dankert-Roelse en Edward Dompeling (MUMC), uiteindelijk resulterend in dit proefschrift. Gedurende die periode was ze gedetacheerd bij het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) in Bilthoven op de laboratorium voor infectieziekten en perinatale screening (LIS) onder leiding van Gerard Loeber en Bert Elvers. Vanaf 2009 heeft ze haar onderzoekswerk gecombineerd met haar werk als waarnemend kinderarts in het Rijnland ziekenhuis te Leiderdorp en het Bovenij ziekenhuis te Amsterdam. Sinds november 2011 is Annette vast werkzaam als kinderarts in het Sint Jansdal ziekenhuis te Harderwijk.
Annette is in 2002 getrouwd met Joris Vernooij. Samen hebben zij 3 kinderen: Merel (2005), Joppe (2008) en Floor (2011).

Dankwoord

Dankwoord

Promoveren is als leren zwemmen. Eerst leer je om je hoofd boven water te houden en gaandeweg wordt je techniek beter en kom je vooruit. Dit boekje is de afronding van zeven jaar werk, en dit stuk is de afsluiting en waarschijnlijk het meest gelezen deel van dit boek.
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Allereerst natuurlijk de ouders en hun pasgeboren kinderen, omdat zij hun kind lieten onderzoeken op CF door middel van de hielprik. Door hun deelname is deze studie een succes geworden.

Dr. J.E. Dankert-Roelse, beste Jeannette, dank voor je vertrouwen dat ik de CHOPIN studie tot een goed einde zou brengen. Ondanks de afstand Utrecht-Heerlen, die het soms best lastig maakte, heb ik veel geleerd. Je eigen proefschrift ging over hetzelfde onderwerp en eindelijk is de neonatale screening op CF in Nederland een feit, nu kan je echt gaan genieten van je pensioen.

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Appendix

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Informatie en vragenlijst focusgroepen

Wat is Cystic Fibrosis (CF, taaislijmziekte)?

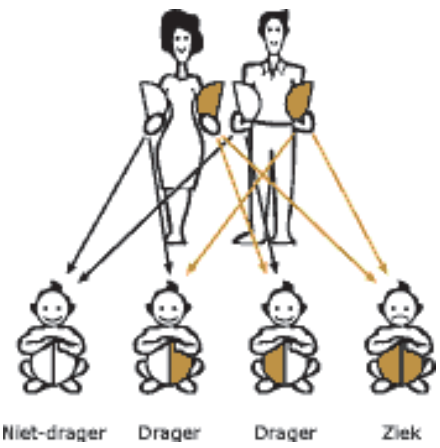
Kinderen met CF (taaislijmziekte) maken dikker en taaier slijm dan normaal. Dit dikke en taaie slijm veroorzaakt problemen in de luchtwegen en in het maagdarmkanaal. Vroege behandeling kan helpen om deze problemen te verminderen. CF (taaislijmziekte) komt voor bij één op de 4750 pasgeborenen.

Erfelijkheid

Als twee mensen samen een kind krijgen, geven ze allebei eigenschappen door aan hun kind. Bijvoorbeeld de kleur van het haar en de kleur van de ogen. We noemen dat erfelijkheid. Een kind erft eigenschappen van zijn vader en van zijn moeder.
Een kind kan ook **ziektes** erven van zijn ouders. CF (taaislijmziekte) is ook een erfelijke ziekte.

Ieder mens heeft erfelijke eigenschappen. Erfelijke eigenschappen zitten in wat we noemen “genen”. Genen zitten in de cellen in het lichaam. Ze zijn heel klein, we kunnen ze niet zien. Iedereen heeft ruim 20.000 genen. Voor erfelijke eigenschappen heeft een mens twee genen: één hebben we gekregen van moeder en de andere van vader. Ook voor het krijgen van CF (taaislijmziekte) hebben we twee genen. Mensen met CF (taaislijmziekte) hebben twee “zieke” genen. Zij hebben dus van hun moeder én van hun vader een “ziek” gen voor de ziekte gekregen.

Drager zijn van CF (taaislijmziekte)



Sommige mensen hebben maar één “ziek” gen voor de ziekte. Het andere gen is dan “gezond”. Deze mensen zijn niet ziek. Ze merken er niets van. Zo iemand met één ziek gen en één gezond gen wordt een **drager** van de ziekte genoemd. Een drager “draagt” de oorzaak van de ziekte (het “zieke” gen), zonder het zelf te merken. Een drager kan het “zieke” gen wel doorgeven aan zijn of haar kinderen.
Als twee dragers samen een kind krijgen, dan kunnen zij allebei het “zieke” gen doorgeven aan hun kind. Het kind heeft dan twee “zieke” genen. Een kind met twee “zieke” genen heeft de ziekte.

Een kind kan dus een erfelijke ziekte, zoals CF (taaislijmziekte), van zijn of haar ouders krijgen terwijl de ouders zelf niet ziek zijn. De ouders zijn dan allebei drager van het “zieke” gen.

De hielprik op CF

Momenteel wordt in de hielprik van baby’s geboren in Zuid-Oost Nederland onderzoek gedaan naar CF. Het voordeel hiervan is dat kinderen met CF die door de hielprik worden opgespoord behandeld kunnen worden vóórdat zij ernstige gezondheidsklachten krijgen.

In het hielprikbloed wordt eerst een simpele test gedaan om te kijken of het kind ***een verhoogde kans*** heeft om CF (taaislijmziekte) te hebben. Als deze simpele test afwijkend is (dit is zo bij ongeveer 2 van de 100 kinderen) wordt een tweede test gedaan.) Bij deze test (de DNA-test) wordt onderzocht of de baby daadwerkelijk CF (taaislijmziekte) heeft. De baby moet dan 2 "zieke" CF (taaislijmziekte) genen hebben. Bij 98 van de 100 kinderen wordt de tweede test niet gedaan. Alleen ouders van wie het kind 2 zieke CF-genen heeft, ontvangen de uitslag dat hun kind zeer waarschijnlijk CF heeft.

De hielprik op CF en dragerschap

Bij de kinderen die de extra DNA test krijgen kan ook eventueel gevonden worden dat ze drager zijn (dus 1 "ziek" gen meedragen). Als een kind drager is van CF (taaislijmziekte) is het gezond.

Als er tijdens een DNA test (die dus uiteindelijk maar bij 2 op 100 kinderen gedaan wordt) gevonden wordt dat het kind drager is, wordt dit momenteel niet meegedeeld aan ouders. Dit is zo besloten omdat dragerschap geen gevolgen heeft voor de gezondheid van de baby. Wel kunnen ouders die willen weten of er een DNA-test is gedaan in het hielprikbloed van hun baby dit bij het laboratorium navragen.

Echter, als het kind dat drager is later kinderen wil krijgen heeft hij/zij een verhoogd risico op een kind met de ziekte CF, als zijn/haar partner toevallig ook drager is. Bovendien kan deze bevinding nu al gevolgen hebben voor de ouders. Het feit dat het kind drager is van CF (taaislijmziekte) betekent namelijk dat ook tenminste één van de twee ouders, drager is van het CF gen. Er is dan een verhoogde kans dat beide ouders drager van het CF gen zijn. Een ouderpaar waarbij beide ouders drager zijn, heeft bij een volgende zwangerschap een sterk verhoogde kans op een kind met CF. Dan is de kans dat een kind van dit ouderpaar CF (2 “zieke” genen) heeft namelijk 1 op 4 (25%),

zie afbeelding. Ouders hebben daarom, als hun kind drager blijkt van het CF-gen, zelf de keuze om te laten onderzoeken of zij zelf ook drager zijn in verband met een volgende zwangerschap.

Samengevat :Bij 2 van de 100 kinderen die een hielprik onderzoek op CF krijgen wordt een DNA test gedaan. Als bij deze test alleen wordt gevonden dat een kind drager is wordt dit niet aan de ouders verteld. Dit is besloten omdat het drager zijn geen gevolgen voor de gezondheid van het kind heeft en omdat bij 98 van de 100 kinderen géén DNA-test wordt gedaan.

Wij willen nu graag weten of u dit een juiste gang van zaken vindt, of dat u liever direct op de hoogte wordt gebracht als in het laboratorium blijkt dat uw kind drager is.

Allereerst willen wij u vragen of u van de onderstaande stellingen wilt aangegeven of ze volgens u waar of niet waar zijn (u kunt het ook aangeven als u het antwoord niet weet):

		Waar	Niet waar	Weet niet
1	CF wordt ook wel taaislijmziekte genoemd omdat er taaier slijm wordt aangemaakt in verschillende delen van het lichaam	❑	❑	❑
2	Iemand die gezond is kan drager zijn van CF	❑	❑	❑
3	Als een kind drager blijkt van CF, dan betekent dit dat in elk geval 1 van zijn ouders ook drager is.	❑	❑	❑
4	Na een eerste simpele test op het hielprikbloed kun je al met zekerheid zeggen of een kind drager is van CF	❑	❑	❑
5	Bij het huidige CF onderzoek in de hielprik krijgen ouders het altijd te horen als hun kind drager blijkt van CF	❑	❑	❑

Nu volgen enkele vragen naar uw mening over het testen op dragerschap van CF in de hielprik voor pasgeborenen

Stel dat in het laboratorium is vastgesteld dat uw kind drager is van CF (taaislijmziekte).

1. Zou u door het laboratorium op de hoogte gesteld willen worden als uit de DNA test van het hielprikbloed blijkt dat uw kind drager van het "zieke" CF gen is (dus 1 ziek gen met zich meedraagt, maar niet ziek is)?

- ❑ Ja
- ❑ Nee

Wat is uw reden hiervoor?

Stel dat in het laboratorium is vastgesteld dat uw kind drager is van CF (taaislijmziekte).

2. Zouden u en/of uw partner dan zelf een dragerschaptest willen doen om te kijken wie van u beiden drager is / zijn (i.v.m. de gevolgen voor een volgende zwangerschap)?

- ❑ Nee
- ❑ Wellicht, ik en/of mijn partner zouden een dragerschapstest overwegen
- ❑ Ja, maar alleen als wij nog een volgende zwangerschapswens hebben
- ❑ Ja, een van ons beiden zou zich dan in elk geval laten testen

Wat is uw reden hiervoor?

3. Zou u uw familie inlichten als blijkt dat uw kind drager is van CF?

- ❑ Nee
- ❑ Ja, alleen directe familieleden (broer, zus, vader, moeder, kinderen)
- ❑ Ja, ik zou alle familieleden voor wie de uitslag relevant zou kunnen zijn inlichten

Wat is uw reden hiervoor?

De situatie op dit moment is dus dat er bij ongeveer 2 op de 100 kinderen een DNA test wordt gedaan en dat ouders alleen bericht krijgen als hun kind CF/taaislijmziekte (2 zieke genen) heeft. Als bij de DNA test blijkt dat de baby drager is van CF (de baby draagt 1 ziek gen mee maar is niet ziek), krijgen ouders geen bericht. Als ouders willen weten of hun baby drager is moeten zij dit zelf navragen.

4. **Vindt u dit een juiste gang van zaken? Of vindt u dat ouders altijd door onderzoekers of de arts ingelicht moeten worden, als er bij hun kind het extra DNA onderzoek gedaan wordt en hun kind vervolgens niet de ziekte blijkt te hebben maar wel drager van CF (taaislijmziekte) blijkt te zijn?**
   Meerdere antwoorden mogelijk

   - ❑ Ik zou het prima vinden als ik geen bericht krijg, als uit de DNA test blijkt dat mijn kind drager is van een CF gen.
   - ❑ Ik zou in ieder geval altijd willen weten dat het **DNA** van het hielprikbloed van mijn kind **getest is op de ziekte CF**.
   - ❑ Ik zou de mogelijkheid willen hebben om op de hielprikkaart aan te geven of ik wel of niet een uitslag wil ontvangen, als uit de DNA test blijkt dat mijn kind drager is van het CF gen
   - ❑ Ik zou het sowieso altijd willen weten als uit de DNA test van mijn kind blijkt dat mijn kind drager is van CF (taaislijmziekte)
   - ❑ Ik heb hier geen mening over

Wat is uw reden hiervoor?

5. **Stel u voor dat uit de DNA test blijkt dat uw kind drager is van een CFgen :**
   Bent u het eens met de opvatting dat dit niet aan ouders gemeld wordt omdat een drager gezond is ?
   - ❑ Ja
   - ❑ Nee

Wat is uw reden hiervoor?

6. Stel u voor dat uit de DNA test blijkt dat uw kind drager is van een CF gen:
   Bent u het eens met de opvatting dat dit niet aan ouders gemeld wordt omdat bij 98 van de 100 kinderen géén DNA-test wordt gedaan?
   Met andere woorden: **er wordt niet in de eerste plaats op dragerschap van CF getest / naar dragers van CF gezocht**

   ❑ Ja
   ❑ Nee

Wat is uw reden hiervoor?

Stelt u zich voor dat het mogelijk is dat ouders geïnformeerd worden over een dragerschapsuitslag op CF in de hielprik

7. **Vindt u dan dat ouders het recht hebben om te kiezen dat zij hier NIET over geïnformeerd willen worden, als uit de hielprik blijkt dat hun kind drager is van CF?**
   Met andere woorden: **Vindt u dat ouders het recht hebben om ergens aan te geven dat zij het NIET willen weten als hun kind drager blijkt?**

   ❑ Ja
   ❑ Nee

Wat is uw reden hiervoor?

TOT SLOT NOG EEN AANTAL VRAGEN OVER UZELF

Dit is om te weten welke ***groep*** mensen deze vragen beantwoord heeft.

1. Hoe oud bent u? ….. jaar

2. Wat is uw geslacht?
   - ❑ Man
   - ❑ Vrouw

3. Wat is de hoogste opleiding die u met een diploma heeft afgesloten?
   (één antwoord aankruisen)
   - ❑ Geen opleiding (lager onderwijs: niet afgemaakt)
   - ❑ Lager onderwijs (basisschool, speciaal basisonderwijs)
   - ❑ Lager of voorbereidend beroepsonderwijs (zoals LTS, LEAO, LHNO, VMBO)
   - ❑ Middelbaar algemeen voortgezet onderwijs (zoals MAVO, [M]ULO, MBO-kort, VMBO-t)
   - ❑ Middelbaar beroepsonderwijs en beroepsbegeleidend onderwijs (zoals MBO-lang, MTS, MEAO, BOL, BBL, INAS)
   - ❑ Hoger algemeen en voorbereidend wetenschappelijk onderwijs (zoals HAVO, VWO Atheneum, Gymnasium, HBS, MMS)
   - ❑ Hoger beroepsonderwijs (zoals HBO, HTS, HEAO, HBO-V, kandidaats wetenschappelijk onderwijs)
   - ❑ Wetenschappelijk onderwijs (universiteit)
   - ❑ Anders, namelijk: ……..

4. Wat is uw burgerlijke staat?

   - ❑ Getrouwd
   - ❑ Geregistreerd partnerschap (samenwonend)
   - ❑ Samenwonend
   - ❑ LAT-relatie
   - ❑ Alleenstaand
   - ❑ Anders, namelijk: ……..

5. Heeft u nog andere kinderen?
   - ❑ Nee
   - ❑ Ja: Zo ja, hoeveel? ……

6. In welk land bent u geboren?
   - ❑ Nederland
   - ❑ Ander land, namelijk: ……

7. Wat zijn de 4 cijfers van uw postcode? ……

Heeft u nog opmerking over dit onderwerp of deze vragenlijst?

HARTELIJK DANK VOOR HET INVULLEN VAN DEZE VRAGENLIJST!
Kunt u nog een keer nagaan of u alle pagina's en vragen heeft ingevuld?

Vragenlijst voorlichting CF onderzoek in hielprik

Hieronder ziet u een aantal uitspraken over het CF onderzoek dat gedaan wordt bij de hielprik bij pasgeborenen in uw regio.
Kunt u van de volgende uitspraken aangeven of ze 'waar' of 'niet waar' zijn?
Als u het antwoord niet weet, dan kunt u 'weet niet' aankruisen.

		Waar	Niet waar	Weet niet
		1	2	3
1.	Tijdige opsporing en vroege behandeling van CF kunnen helpen om de problemen die bij CF horen te verminderen.	❑	❑	❑
2.	De kans dat een kind CF heeft is heel klein (kleiner dan 1%).	❑	❑	❑
3.	Deelname aan het CF onderzoek is verplicht voor elke pasgeboren baby.	❑	❑	❑
4.	Voor het onderzoek naar CF is meer bloed nodig en daarom moet er extra bloed geprikt worden bij de hielprik.	❑	❑	❑
5.	Alle baby's met een afwijkende uitslag van het CF onderzoek in de hielprik hebben daadwerkelijk CF	❑	❑	❑
6.	U ontvangt OOK bericht als de uitslag van het onderzoek GOED is.	❑	❑	❑
7.	De gegevens mogen uitsluitend worden gebruikt voor het onderzoek naar CF en mogen dus niet voor onderzoek aan anderen worden gegeven.	❑	❑	❑
8.	Iemand die gezond is kan drager zijn van CF.	❑	❑	❑
9.	Als de uitslag van het CF onderzoek in de hielprik 'afwijkend' is, dan zal de huisarts contact met u opnemen.	❑	❑	❑
10.	Bij het CF onderzoek krijgt u ook altijd te horen of uw kind drager is van CF.	❑	❑	❑
11.	Mijn kind heeft de ziekte CF	❑	❑	❑

Wij willen nu graag uw mening weten over het CF onderzoek in de hielprik.
Kunt u bij de volgende vragen bij elke vraag het hokje aankruisen dat het meest overeenkomt met uw antwoord?:

12. Hoe groot schat u de kans in dat uw kind de ziekte CF heeft?

	1	2	3	4	5	
Kans is niet aanwezig	❑	❑	❑	❑	❑	Kans is zeer groot

13. Hoe betrouwbaar denkt u dat de CF test in de hielprik is?

	1	2	3	4	5	
Niet betrouwbaar	❑	❑	❑	❑	❑	Wel betrouwbaar

14. Heeft u spijt van uw deelname aan het CF onderzoek in de hielprik?

	1	2	3	4	5	
Veel spijt	❑	❑	❑	❑	❑	Geen spijt

15. Zou u bij een eventuele volgende zwangerschap weer meedoen aan het CF onderzoek in de hielprik?

	1	2	3	4	5	
Zeker niet	❑	❑	❑	❑	❑	Zeker wel

16. Bent u voldoende geïnformeerd over het CF onderzoek in de hielprik?
(meerdere antwoorden mogelijk)

1. ❑ Ja, de informatie was goed, duidelijk en volledig
2. ❑ Nee, ik had meer informatie willen hebben over waarom CF is toegevoegd aan de hielprik in mijn regio
3. ❑ Nee, ik had meer informatie willen krijgen over wat de ziekte CF precies inhoudt
4. ❑ Nee, ik had meer informatie willen krijgen over dragerschap / erfelijkheid van CF
5. ❑ Ik had meer informatie willen hebben over de mogelijkheid dat na een "afwijkende" uitslag er vervolgonderzoek kan plaatsvinden.
6. ❑ Nee, ik had eerder informatie willen krijgen
7. ❑ Nee, ik vind dat ik tevéél informatie heb ontvangen
8. ❑ Anders, namelijk: .....

17. Welk rapportcijfer zou u geven voor de voorlichting die u heeft gekregen over het CF onderzoek in uw regio?

Hieronder staan vier manieren waarop u voorlichting over de hielprik kunt hebben gehad. Geeft u elke manier een rapportcijfer. Als u niet op de genoemde manier bent voorgelicht, dan kunt u dat ook aangeven.

De rapportcijfers lopen van 1 ("zeer slecht") tot 10 ("uitmuntend"). Het cijfer 6 betekent "voldoende". Alle cijfers lager dan 6 zijn onvoldoende, te beginnen met het cijfer 5,5. U kunt dus ook "halven" geven, zoals 7,5.

Voorlichting door:

a.	verloskundige	.....	cijfer
		❑	geen voorlichting gekregen
b.	extra CF folder bij hielprik folder	.....	cijfer
		❑	geen CF folder gekregen
c.	website hielprik van het RIVM	.....	cijfer
		❑	niet op de website gekeken
d.	zorgverlener die de hielprik heeft afgenomen	.....	cijfer
		❑	geen voorlichting gekregen

18. Heeft u op dit moment nog vragen over het CF onderzoek bij de hielprik?

	1	2	3	4	5	
Veel vragen	❑	❑	❑	❑	❑	Geen vragen

Welke **vragen** heeft u nog over het CF onderzoek in de hielprik?

De onderstaande vragen gaan over allerlei dingen die te maken hebben met de gezondheid van uw kind. U kunt de vragen beantwoorden door het antwoord aan te kruisen dat het beste bij uw kind past.

Had uw kind de afgelopen 3 maanden last van

		1 nooit	2 soms	3 vaak
19.	maag- of buikpijn?	❑	❑	❑
20.	krampjes?	❑	❑	❑
21.	moeite met ademhaling of longproblemen?	❑	❑	❑
22.	benauwdheid?	❑	❑	❑

Hoe sliep uw kind de afgelopen 3 maanden?

		1 nooit	2 soms	3 vaak
23.	Sliep uw kind onrustig?	❑	❑	❑
24.	Huilde uw kind 's nachts?	❑	❑	❑

Hoe at en dronk uw kind in de afgelopen 3 maanden?

		1 nooit	2 soms	3 vaak
25.	Had uw kind een slechte eetlust?	❑	❑	❑

Hoe is uw kind's gezondheid in het algemeen?

26. Hoe vaak is uw baby ziek, in vergelijking met andere baby's
    1. ❑ Vaker
    2. ❑ Even vaak
    3. ❑ Minder vaak

27. Hoe vaak bent u in de afgelopen maand met uw kind naar de huisarts geweest?

1. ❑ Niet
2. ❑ 1 keer
3. ❑ 2-3 keer
4. ❑ > 4 keer

28. Hoe bezorgd bent u momenteel over de gezondheid van uw kind?

	1	2	3	4	5	
Niet bezorgd	❑	❑	❑	❑	❑	Wel bezorgd

Hoe was uw kind de afgelopen 3 maanden?

		1 nooit	2 soms	3 vaak
29.	Blij	❑	❑	❑
		nooit	soms	vaak
30.	Gelukkig	❑	❑	❑
		nooit	soms	vaak
31.	Mijn kind was opstandig/dwars tegen mij	❑	❑	❑

Hieronder vindt u een aantal vragen die betrekking hebben op hoe u zich voelt. Kruis bij elke vraag het antwoord aan dat het beste weergeeft hoe u zich gedurende ***de afgelopen week*** heeft gevoeld. Probeer niet te lang na te denken over uw antwoord.

32. Ik voel me gespannen:

1. ❑ Meestal
2. ❑ Vaak
3. ❑ Af en toe, soms
4. ❑ Helemaal niet

33. Ik kan rustig zitten en me ontspannen:
    1. ❑ Zeker
    2. ❑ Meestal
    3. ❑ Niet vaak
    3. ❑ Helemaal niet

34. Ik maak me vaak ongerust:
    1. ❑ Heel erg vaak
    2. ❑ Vaak
    3. ❑ Af en toe maar niet te vaak
    4. ❑ Alleen soms

35. Ik voel me opgewekt
    1. ❑ Helemaal niet
    2. ❑ Niet vaak
    3. ❑ Soms
    4. ❑ Meestal

Kunt u bij de volgende vragen het hokje aankruisen dat het beste uw antwoord omschrijft:

36. Wist u dat het bloed van uw kind op CF onderzocht zou worden op het moment dat de hielprik bij uw kind gedaan werd?
    1. ❑ Ja
    2. ❑ Nee

U bent door uw huisarts (of de kinderarts in ziekenhuis) geïnformeerd over de eerste uitslag van de hielprik. Dit is gebeurd kort nadat de hielprik is afgenomen.

37. Hoe tevreden bent u over de manier waarop uw huisarts/kinderarts u heeft geïnformeerd over de uitslag van de hielprik?
    1. ❑ Tevreden
    2. ❑ Beetje tevreden
    3. ❑ Niet tevreden, niet ontevreden
    4. ❑ Beetje ontevreden
    5. ❑ Ontevreden

38. Heeft u het idee dat uw huisarts/kinderarts voldoende kennis had over CF?
    1. ❑ Ja
    2. ❑ Een beetje
    3. ❑ Nee

39. Nam de huisarts/kinderarts voldoende tijd om uw vragen te beantwoorden?
    1. ❑ Ja
    2. ❑ Een beetje
    3. ❑ Nee

40 Hoeveel tijd zat er tussen het eerste bericht dat u van uw huisarts (of kinderarts in het ziekenhuis) kreeg over de afwijkende uitslag voor CF in de hielprik en de afspraak voor het vervolgonderzoek (de zweettest)?
    1. ❑ 0 dagen (de zweettest vond plaats op de dag dat u werd geïnformeerd over de afwijkende uitslag)
    2. ❑ 1 dag
    3. ❑ 2 dagen
    4. ❑ 3 dagen
    5. ❑ Anders, namelijk: .....

41 Wat vindt u van de tijdsduur die er zat tussen het bericht dat u van de huisarts (of kinderarts in het ziekenhuis) kreeg over de afwijkende uitslag voor CF in de hielprik en de afspraak voor de zweettest?
    1. ❑ Te kort
    2. ❑ Precies goed
    3. ❑ Te lang

42. Heeft u na de uitslag van de hielprik over de uitslag gepraat met uw familie, vrienden en/of buren?

	1	2	3	4	5	
Helemaal niet	❑	❑	❑	❑	❑	Vaak

43. Kon u bij mensen uit uw omgeving terecht voor steun?

	1	2	3	4	5	
Helemaal niet	❑	❑	❑	❑	❑	Vaak

44. Heeft u na de uitslag van de hielprik op CF nog verdere informatie gezocht? (meerdere antwoorden mogelijk)

1. ❑ Ja, via de website over het CF onderzoek bij de hielprik van het RIVM of NCFS
2. ❑ Ja, via het internet naar andere websites en informatie over CF gezocht
3. ❑ Ja, ik heb andere folders/boeken/tijdschriften/artikelen gelezen
4. ❑ Ja, ik ben met anderen gaan praten
5 ❑ Ja, ik heb op video/DVD informatie gezien
6. ❑ Ja, ik heb vragen gesteld aan de verloskundige, screener of huisarts
7. ❑ Ja, maar op een andere manier, namelijk .....
8. ❑ Nee, ik heb geen verdere informatie gezocht

Kunt u hieronder aangeven hoe u zich voelde na het horen van de eerste "afwijkende" uitslag op CF nadat de hielprik bij uw kind was uitgevoerd

		1	2	3	4	5	
45.	Geschrokken	❑	❑	❑	❑	❑	Niet geschrokken
46.	Bezorgd	❑	❑	❑	❑	❑	Niet bezorgd
47.	Angstig	❑	❑	❑	❑	❑	Niet angstig
48.	Ongelukkig	❑	❑	❑	❑	❑	Niet ongelukkig
49.	Gerustgesteld	❑	❑	❑	❑	❑	Niet gerustgesteld
50.	Opgelucht	❑	❑	❑	❑	❑	Niet opgelucht

Vervolgens is er na de uitslag op de hielprik vervolgonderzoek gedaan bij uw kind in het ziekenhuis.

51. Hoe tevreden bent u over de informatie, het onderzoek en de begeleiding tijdens het vervolgonderzoek in het ziekenhuis?
    1. ❑ Tevreden
    2. ❑ Beetje tevreden
    3. ❑ Niet tevreden, niet ontevreden
    4. ❑ Beetje ontevreden
    5. ❑ Ontevreden

52. Werd er tijdens het vervolgonderzoek in het ziekenhuis voldoende tijd genomen om uw vragen te beantwoorden?
    1. ❑ Ja
    2. ❑ Een beetje
    3. ❑ Nee

53. Hoeveel tijd zat er tussen de afspraak van de zweettest en de uitslag van de zweettest?
    1. ❑ De zweettest was mislukt en moest herhaald worden
    2. ❑ 0 dagen (u kreeg op de dag van de zweettest ook de uitslag van de zweettest)
    3. ❑ 1 dag
    4. ❑ 2 dagen
    5. ❑ 3 dagen
    6. ❑ Anders, namelijk: .....

54. Wat vindt u van de tijdsduur die er zat tussen de afspraak van de zweettest en de uitslag van de zweettest?
    1. ❑ Te kort
    2. ❑ Precies goed
    3. ❑ Te lang

Kunt u hieronder aangeven hoe u zich voelde na het horen van de uiteindelijke uitslag op CF nadat er vervolgonderzoek gedaan is in het ziekenhuis

		1	2	3	4	5	
55.	Geschrokken	❑	❑	❑	❑	❑	Niet geschrokken
56.	Bezorgd	❑	❑	❑	❑	❑	Niet bezorgd
57.	Angstig	❑	❑	❑	❑	❑	Niet angstig
58.	Ongelukkig	❑	❑	❑	❑	❑	Niet ongelukkig
59.	Gerustgesteld	❑	❑	❑	❑	❑	Niet gerustgesteld
60.	Opgelucht	❑	❑	❑	❑	❑	Niet opgelucht

TOT SLOT NOG EEN PAAR VRAGEN OVER UZELF

Dit is om te weten welke ***groep*** mensen deze vragen beantwoord heeft.

61. Hoe oud bent u? ….. jaar

62. Door wie is deze vragenlijst ingevuld?
    - ❑ Moeder
    - ❑ Vader
    - ❑ Anders, namelijk: ……..

63. Wat is de hoogste opleiding die u met een diploma heeft afgesloten?
    (één antwoord aankruisen)
    - ❑ Geen opleiding (lager onderwijs: niet afgemaakt)
    - ❑ Lager onderwijs (basisschool, speciaal basisonderwijs)
    - ❑ Lager of voorbereidend beroepsonderwijs (zoals LTS, LEAO, LHNO, VMBO)
    - ❑ Middelbaar algemeen voortgezet onderwijs (zoals MAVO, [M]ULO, MBO-kort, VMBO-t)
    - ❑ Middelbaar beroepsonderwijs en beroepsbegeleidend onderwijs (zoals MBO-lang, MTS, MEAO, BOL, BBL, INAS)
    - ❑ Hoger algemeen en voorbereidend wetenschappelijk onderwijs (zoals HAVO, VWO Atheneum, Gymnasium, HBS, MMS)
    - ❑ Hoger beroepsonderwijs (zoals HBO, HTS, HEAO, HBO-V, kandidaats wetenschappelijk onderwijs)
    - ❑ Wetenschappelijk onderwijs (universiteit)
    - ❑ Anders, namelijk: ……..

64. Wat is uw burgerlijke staat?
- ❑ Getrouwd
- ❑ Samenwonend
- ❑ Alleenstaand
- ❑ Anders, namelijk: .....

65. Heeft u nog andere kinderen?
- ❑ Nee
- ❑ Ja: Zo ja, hoeveel? .....

66. In welk land bent u geboren?
- ❑ Nederland
- ❑ Ander land, namelijk: .....

67. Welke taal spreekt u thuis?
- ❑ Alleen Nederlands
- ❑ Nederlands en een andere taal
- ❑ Andere taal, namelijk: .....

68. Kunt u zich in het Nederlands verstaanbaar maken?
- ❑ Nee, ik spreek geen Nederlands
- ❑ Ja, maar met veel moeite
- ❑ Ja, maar met enige moeite
- ❑ Ja, ik spreek goed Nederlands

69. Heeft u moeite met het begrijpen van Nederlands?
- ❑ Ja, altijd
- ❑ Ja, vaak
- ❑ Ja, soms
- ❑ Nee, nooit

70. Heeft u moeite bij het lezen van kranten, brieven of folders in het Nederlands?
- ❑ Ja, altijd
- ❑ Ja, vaak
- ❑ Ja, soms
- ❑ Nee, nooit

71. Heeft uw baby gezondheidsproblemen. Zo ja, welke?
    - ❑ Nee
    - ❑ Ja, namelijk: .....

72. Krijgt uw baby medicijnen voor gezondheidsproblemen? Zo ja, welke?
    - ❑ Nee
    - ❑ Ja, namelijk: .....

73. Wat zijn de 4 cijfers van uw postcode? ……

Heeft u nog **opmerkingen** over deze vragenlijst of over de hielprik, dan kunt u deze hieronder invullen.

HARTELIJK DANK VOOR HET INVULLEN VAN DEZE VRAGENLIJST!
Kunt u nog even controleren of u alle pagina's en vragen heeft ingevuld?

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Abbreviations

ATP	adenosine-5'-triphosphate
AUC	area under the curve
AZL	Leiden University Medical Centre (LUMC)
C	control
CD	clinical diagnosis
CER	cost-effectiveness ratio
CF	cystic fibrosis
CFF	Cystic Fibrosis Foundation
CFTR	cystic fibrosis transmembrane regulator
CHOPIN	Cystic fibrosis Heelprick amOng a newborn Population In the Netherlands
CI	confidence interval
CPB	College of Protection of Personal data
CVB	Centre for Population Research
DELFIA	dissociation-enhanced lanthanide fluorescent immunoassay
DGGE	denaturing gradient gel electrophoresis
DNA	desoxyribonucleic acid
DPSU	Dutch Paediatric Surveillance Unit
EGA	extended gene analysis
ELISA	enzyme-linked immunosorbent assay
EMC	Erasmus Medical Centre
FP	false-positive
GA	gestational age
GP	general practitioner
HADS	Hospital Anxiety and Depression Scale
ICM	intestinal current measurements
IQR	interquartile range
IRT	immunoreactive trypsinogen
ISNS	International Society for Neonatal Screening
MCADD	medium chain acyl-CoA dehydrogenase deficiency
MI	meconium ileus
mRNA	messenger ribonucleic acid
MSD	membrane spanning domain
MUMC	Maastricht University Medical Centre
NBD	nucleotide binding domain
NBS	newborn screening
NBSCF	newborn screening for cystic fibrosis

NCFS	Dutch cystic fibrosis foundation
NVK	Dutch paediatric association
OR	odds ratio
PAP	pancreatitis-associated protein
PBS	phosphate buffered saline
PI	pancreas insufficient
PPV	positive predictive value
PS	pancreas sufficient
QPIT	quantitative pilocarpine ionthophoresis test
RIVM	National Institute for Public Health and the Environment
ROC	receiver operating characteristic curve
SD	standard deviation
SDS	standard deviation score
Sens	sensitivity
Seq	sequencing
Spec	specificity
SPSS	Statistical Package for the Social Sciences
TAPQOL	TNO-AZL Preschool children Quality Of life Questionnaire
TNO	Netherlands Organisation for Applied Scientific Research
UK	United Kingdom
UMCN	University Medical Centre Nijmegen-St Radboud
UMCU	University Medical Centre Utrecht
US	United States
WHO	World Health Organisation
WKZ	Wilhelmina children's hospital, UMC Utrecht
ZonMw	The Netherlands Organisation for Health Research and Development